MICROARRAYS: CHIPPING AWAY AT THE MYSTERIES OF CHICKEN GENOMICS

T.M. CROWLEY\textsuperscript{1,2} and R.J. MOORE\textsuperscript{1,2}

\textbf{Summary}

Our knowledge of avian genomics has rapidly increased over the past few years, culminating in the recent publication of the chicken genome sequence and the development of several microarray platforms to study gene expression. Microarrays have enabled chicken biologists to investigate the expression of thousands of genes across many conditions, including genetic control of development, cellular differentiation, and adaptation to biological challenges. Thus, it is easy to see why microarrays have such a vast potential in not only chicken biology but the entire agricultural sector. It is anticipated that the continued development of microarray technology will be paralleled with improvements in the health and productivity of chickens.

\section*{I. INTRODUCTION}

From their origins in Southwest Asia, chickens have spread to all corners of the world due in great part to their usefulness to man. There is an ever increasing demand for chicken meat. The ability of producers to meet this demand has been assisted by an approximate halving in typical production time to marketable weight in the past 50 years. This huge time reduction is due to both traditional breeding methods and improved management strategies. The rate of improvement is still being maintained and with the advent of new molecular based technologies it is sure to continue.

Microarrays are tools that allow exploration and discovery at a genomic level. Essentially, microarrays are assays that can be used to concurrently measure the expression of thousands of genes. Microarrays are often used to capture a "molecular portrait" of the living cell or tissue at the moment of sampling. By parallel comparison of the portrait among samples of different physiological or pathological origin, a molecular signature can emerge which is characteristic of the different state. This information then facilitates a higher-level understanding of the physiology or pathology state of the organism thus offering insights into fundamental aspects of cell biology. These microscopic arrays of large sets of DNA sequences, together with the recently obtained knowledge of the chicken genome, have the potential to revolutionise chicken biology.

\section*{II. THE CHICKEN GENOME}

The chicken is the first bird and first production animal to have its genome sequenced (Hillier \textit{et al.}, 2004). It is an important non-mammalian model organism particularly for embryological studies. While knowledge of the chicken genome will not provide immediate solutions to current poultry problems, such as avian influenza, it has opened the door to a range of promising research that is sure to impact the industry in the coming years. So far, the chicken genome has expanded the number of known or predicted genes from approximately 13,000 previously identified to over 18,000 (Burt, 2004). Included in the most recent set are a large number of immune related genes not previously described in chickens (Burt, 2004). These genes are likely to play a role in many of the infectious diseases that

\textsuperscript{1} Australian Animal Health Laboratories, CSIRO, Geelong, VIC, 3220.
\textsuperscript{2} Australian Poultry CRC, Armidale, NSW, 2350.
threaten poultry production. Currently many diseases are controlled with vaccinations and antibiotics. These current treatments often have limitations and new methods of control need to be investigated. The chicken genome has the potential to play a key role in unravelling ways to improve current disease control methods such as vaccines (Dhiman et al., 2002) and also to identify new ones, by allowing a more comprehensive understanding of how the chicken responds to pathogenic challenges. In addition, this wealth of knowledge together with microarray technology will allow chicken biologists to gain an enhanced understanding of the biology of a chicken at a gene specific level. Regardless of whether the aim is to enhance current vaccines, or develop new disease control strategies, knowledge of the chicken genome is undoubtedly a powerful tool to study immune competence in birds.

III. BASIC PRINCIPLES OF MICROARRAYS

The majority of the cells in the chicken contain the same set of chromosomes and identical genes. The phenotypic differences among cells of different types are determined largely by the level of expression of the genes. Some genes may be switched on, ‘expressed’ in one cell type and switched off ‘not expressed’ in a different cell type. Essentially microarrays measure this gene expression, which is a term used to describe the transcription of information contained in the DNA into messenger RNA (mRNA) molecules that are translated into proteins, which in turn perform the most critical functions of cells. The study of mRNA produced by a cell, including the various types and their amounts, allows insight into what genes are being expressed. The assumption is made that in general mRNA levels give a good overall indication of the amount of the equivalent encoded protein. Gene expression is a dynamic and highly regulated process that enables a cell to respond to environmental stimuli and to its own changing needs. Normal cell conditions see a harmonious expression of a range of different genes that are critical to normal growth, development and function. Disruptions or changes to this gene expression equilibrium may result in disease.

Microarrays typically consist of a small glass slide onto which hundreds to thousands of DNA templates are attached (spotted) to known locations. This ‘spotting’ can be done by a contact pin printing robot, ink-jet printing, or by photo-lithographic synthesis (similar to production of computer chips) and other in-situ synthesis methods (Dhiman et al., 2002). At present there are a few different types of chicken arrays available including a cDNA (Figure 1) and oligonucleotide array produced by the International Chicken Consortium and a commercial Affymetrix GeneChip® array.

Microarray technology works by exploiting the ability of an mRNA to specifically bind to the template DNA from which it originated. However, the real power of microarrays lies in their ability to screen thousands of DNA templates (spots) in a single experiment and thus gain a snapshot of the gene expression within a cell. There are two main types of microarray experiments, dual colour and single colour. For a dual colour experiment RNA from cells from two different conditions are reverse transcribed to produce complimentary DNA (cDNA). The cDNA is then labelled with two different fluorescent dyes: for example a red dye (Cy5) for the first condition and a green dye (Cy3) for the second condition. The labelled cDNA from both conditions are then hybridised to the microarray, allowing labelled gene products to bind to their complementary sequence (spot) present on the microarray (Figure 2). The attached fluorescent dyes enable the amount of bound cDNA to be measured: for example if cDNA from condition one is in abundance then the spot will appear red, or if the cDNA from the second condition is in abundance the spot will be green and if the cDNA from both conditions is present in equal amounts then the spot will appear yellow (a mixture of red and green) (Xiang and Chen, 2000). Single colour microarrays are in principle the
same as dual colour microarrays, however, they use RNA from a single condition and compare results across slides.

Figure 1. A 13,000K cDNA array (Cogburn et al., 2003) hybridised with a control and an infected sample labelled with Cy5 and Cy3 respectively (Our laboratory). Physical dimensions of this array are 54 mm x 18 mm.

Figure 2. Overview of the experimental steps in a dual colour microarray experiment.

The measurement of the fluorescent intensities (colours) from each spot is obtained using an image scanner (Xiang and Chen, 2000). The raw output microarray data from the image scanner is used to obtain the relative expression levels of the genes in both samples. Each spot on the array is identified and quantified using image analysis software that compares the spot intensity to the background. Following this the data is normalised and a host of statistical tests are applied to determine what genes are being significantly expressed in both conditions. At present there are many programs available to perform the statistical analysis of microarray data, each with different analyses. The rapid development of microarray technology has resulted in the absence of established standards for detection of differentially expressed genes or even a standard unit for what determines gene expression levels. It is for this reason that microarrays in their current form are sometimes regarded as not a stand alone experiment and results are often confirmed using other methods such as quantitative PCR (Q-PCR; Beckman et al., 2004).

Microarrays are not exclusively used to measure gene expression. Another application is the detection of single nucleotide polymorphisms (SNPs), in a gene sequence.
SNP microarrays are spotted with known target sequences usually from a single gene, with each spot differing by only one or a few specific nucleotides (Jalving et al., 2004). A further application of microarrays is comparative genomic hybridisation (CGH) (Muller, 2001). Each of the spots on a CGH microarray contain large pieces of genomic DNA which have a known chromosomal location and the hybridisation mixture has fluorescently labelled DNA harvested from two different conditions: for example DNA from normal and diseased tissue. These microarrays detect changes in the number of copies of a particular gene involved in a disease state: for example growth of a tumour. Single nucleotide polymorphisms can have powerful uses in genetic mapping experiments aimed at defining the genetic causes of superior trait characteristics. The adaptation of microarrays to analyse SNP’s plus the vast number of new SNP’s identified in an adjunct project to the genome sequencing effort, gives poultry breeders very powerful new capabilities to use in the improvement of chicken strains.

IV. CURRENT POULTRY MICROARRAY APPLICATIONS

Microarrays are increasingly being used in the world of poultry research. To date this technology has been used for a handful of applications, including response of cultured chicken cells to Marek’s disease virus (MDV) (Morgan et al., 2001), where they found that MDV infection was linked to expression of TSA-1, a gene important for T-cell differentiation and activation. In addition, differential gene expression between hypothyroid and hyperthyroid chickens and the developing liver has been detected using specific cDNA arrays (Cogburn et al., 2003). A recent microarray study focusing the developing thymus tissue (Cui et al., 2004), has provided an initial profile of the developmental patterns of expression of genes important in the chicken immune system. Microarrays have also been utilised to explore gene expression differences between a range of Broiler chickens including malabsorption syndrome resistant and susceptible lines (van Hemert et al., 2004) and to investigate responses to chemical treatments on the expression of activated T-cells (Kampa and Burns, 2002). Microarrays have also been utilised in a number of different bird species including turkey (Munir and Kapur, 2003; Karaca et al., 2004) and quail (Mott and Ivarie, 2004). Our laboratory is currently investigating chicken gene expression in response to a number of infectious agents including chicken anaemia virus, Marek’s disease virus, Mycoplasma and several gut related pathogens. Through these studies we hope to gain a superior understanding of the chicken’s response to pathogenic challenges, in particular the innate immune response and begin to enhance this response to a range of infectious diseases.

V. THE FUTURE OF MICROARRAYS

While microarrays are still in their infancy it is easy to extrapolate ideas on the future uses of this technology. In addition to the enhancement of treatment and control of poultry diseases this technology could potentially aid diagnosis. It is plausible that in the future poultry farmers may be able to use a simple hand held device to quickly diagnose diseases. Microarrays may also play a prospective role in management and breeding for specific traits or gene profiles. Regardless of what the crystal ball holds for microarrays it is evident that over the coming years this area of technology is sure to be challenging, exciting and fruitful.

VI. CONCLUSION

For many years biologists have had the ability to investigate expression of a small number of genes in a small number of conditions, however, with the advent of microarray technology it is now possible to explore thousands of genes across many conditions. Thus, it
is easy to see why microarrays have such a vast potential in not only chicken biology but the entire agricultural sector. In the case of poultry this potential has been accelerated by the recent sequencing of the chicken genome. This information will complement the information obtained from microarrays and allow chicken biologists to gain a greater understanding of genetic control of development, cellular differentiation, and adaptation to biological challenges. It is anticipated that the continued development of microarray technology will be paralleled with improvements in the health and productivity of chickens.

REFERENCES