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Transcriptomics Research in Chicken

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Abstract: The chicken (*Gallus gallus*) is an important model organism in genetics, developmental biology, immunology and evolutionary research. Moreover, besides being an important model organism the chicken is also a very important agricultural species and an important source of food (eggs and meat). The availability of the draft chicken genome sequence provided many possibilities to in detail study a variety of genomic changes during evolution using a comparison between chicken and mammals. For example, compared to mammals, the use of a Z/W sex determination system is a special aspect of the avian genome where the female is the heterogametic sex (ZW) and the male is the homogametic (ZZ) sex. A comparison of the genomic sequences of platypus, chicken and human showed that sex chromosomes evolved separately in birds and mammals.

Key words: Chicken, transcriptomics, genome, mammals, feed, genetics

INTRODUCTION

The sequence of the chicken genome has provided new possibilities to study the function of the individual genes and gene networks in chicken and to gain insight in their specific roles in chicken physiology. An important challenge in the post-sequence era of chicken biology is determining the functional role of known genes. Currently, the function of many of the chicken genes has been predicted based on the sequence homology to genes of known function in other species. However, because of the differences in physiology among different species it is essential to improve this and to obtain additional functional data in the chicken itself, for example, more detailed information about the expression of these genes in different tissues and under different conditions.

Before 2003, only a few papers on gene expression profiling using microarrays were published in chicken (Cogburn *et al*., 2007). The first picture of global gene expression in the immune system in chicken was provided by lymphoid cDNA microarrays (Neiman *et al*., 2001). Subsequently, several tissue-specific cDNA microarrays were developed and used for transcription profiling in the liver, intestine and bursa of Fabricius (Cogburn *et al*., 2003; Neiman *et al*., 2003; Van Hemert *et al*., 2003). The first high-density (13K) multi-tissue chicken cDNA array to be developed (Burnside *et al*., 2005) was based on ESTs/cDNA clones representing 24 different adult or embryonic tissues. The coverage of cDNA microarray platforms increased very fast during the following years and at the same time, the quality of array manufacturing was also improved. The availability of a draft chicken genome sequence in 2004, made it possible to manufacture whole genome oligo-arrays to study genome-wide gene expression in chicken.

The Chicken Genome GeneChip containing probes for 33,457 chicken and viral pathogen transcripts is commercially available from Affymetrics (http://www.affymetrix.com) (GEO Accession: GPL3213) and this array was the first genome-wide gene expression chip on the market. Microarrays consisting of long oligonucleotides (70 mer) were developed by a number of different groups including the Roslin Institute (ARK-Genomics *G. gallus* 20K; GEO accession: GPL5480, GPL8862), the University of Arizona (*Gallus gallus* 20.7K Oligo Array; GEO accession: GPL6049) and the University of Missouri (*Gallus gallus* 21K; GEO accession: GPL5618). Two of these microarrays are available from the University of Arizona (http://www.grl.steelecenter.arizona.edu/) and

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Table 1: An overview of genome-wide expression studies in chicken in NCBI GEO database

Platform accession ID	Description	Platform name	No. of dataset	No. of arrays
GPL3213	GeneChip	Affymetrix Chicken Genome Array	28	404
GPL4993	Agilent 44K oligo set	Chicken 44K custom Agilent microarray	3	60
GPL7399	-	Agilent custom 44K chicken array	1	20
GPL5480	Roslin/Ark 20K oligo set	ARK-Genomics *G. gallus* 20K v1.0	2	64
GPL5618	-	Missouri *Gallus gallus* 21k	1	9
GPL6049	-	Arizona *Gallus gallus* 20.7K Oligo Array v1.0	2	120
GPL8199	-	ChickenOligo 20.6K 70-mer microarray v2	2	16

ARK Genomics (http://www.ark-genomics.org/). Recently, a chicken 44K custom Agilent microarray (GEO Accession: GPL4993, GPL7399, GPL8764) was developed which currently is available from Agilent (http://www.agilent.com/).

By the time, the experiments for this thesis were carried out, the Agilent platform was not available yet and the cost of chicken 20K oligo-arrays was much lower than the Affymetrix chips. This made it a preferable platform to use within the project because of the relatively large number of samples to survey. Now a days, these commercial long-oligo arrays and the chicken genome array (Gene chip) are increasingly replacing custom microarrays (for example, tissue specific cDNA microarrays) because of the higher standardization and higher quality of these platforms. The current publicly available transcriptomic data in chicken using genome-wide oligo array is shown in Table 1.

The number of genome-wide transcription profiling experiments in chicken has increased dramatically in recent years because of the availability of the microarray platforms described earlier. The availability of these new platforms of improved quality and higher probe coverage, for example the Agilent 44K chicken array, researchers expect that the number of genome-wide transcription profiling studies using these platforms will increase in the near future as well studies that use direct sequence-based technology to study gene expression.

The majority of earlier microarrays studies described in GEO or other publications (Desert *et al*., 2008; Eisenberg and Levanon, 2003; Kim *et al*., 2008a, b; Morgan *et al*., 2001; Van Hemert *et al*., 2006, 2007; Wang *et al*., 2007) were designed to monitor changes of gene expression between different conditions, treatments or time points in a single tissue. The identified candidate genes were subsequently used to try to interpret the underlying biological processes by looking at the functions of these genes. There are many candidate genes identified that lack any functional annotation in the current chicken genome build, hampering the interpretation of the results obtained within these microarray experiments.

This limitation of the current microarray analysis motivated the generation of transcriptional profiling across a number of tissues in project described in this thesis. The global expression pattern of the genes under normal conditions among tissues can be used as an reference baseline for expression studies aimed to study specific diseases in chicken. It provides information about the distribution of the gene transcription profile across an range of tissues under normal conditions and this will facilitate the inference of possible biological functions of un-annotated genes in chicken. Genome-wide gene expression information in chicken can also be used to shed light on other aspects of vertebrate genome and transcriptome evolution. For example, it has been reported in human that housekeeping genes have relatively shorter introns, untranslated regions and coding sequences, suggesting a selection for compactness. With genome-wide gene expression data in chicken across a number of tissues, researchers can identify genes with housekeeping functions and test whether the compactness of housekeeping gene found in human is also true in chicken.

Furthermore, evolutionary changes in gene expression account for most phenotypic differences between different species. Global gene expression patterns were reported to be conserved between human and apes (Khaitovich *et al*., 2006) as well as between human and mouse (Vinogradov, 2004).

CONCLUSION

The results of these studies suggested that the gene expression within mammals is under evolutionary constraint. Comparing gene expression of birds and mammals would help to further understand the gene expression conservation in vertebrates during evolution.

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