Differentially expressed transcripts in shell glands from low and high egg production strains of chickens using cDNA microarrays

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Abstract

We have constructed a tissue-specific in-house cDNA microarray to identify differentially expressed transcripts in shell glands from low (B) and high (L2) egg production strains of Taiwanese country chickens during their egg-laying period. The shell gland cDNA library was constructed from the high egg production strain. cDNA clones (7680) were randomly selected and their 5'-end sequences characterized. After excluding overlapping sequences, an in-house cDNA microarray, representing 2743 non-redundant transcripts, was generated for functional genomic studies. Using our microarray, we have successfully identified 85 differentially expressed transcripts from the two different strains of chicken shell glands. In this study, 34 of these transcripts were associated with signal transduction, protein biosynthesis,

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cell adhesion, cellular metabolism, skeletal development, cell organization and biogenesis. We selected a number of the differentially expressed transcripts for further validation using semi-quantitative RT-PCR. These included elongation factor 2 (EEF2), ovocalyxin-32 (OCX-32) and annexin A2 (ANXA2) which were expressed at high levels in the chicken shell glands of the B strain and, in contrast, the coactosin-like protein (COTL1), transcription factor SOX18 and MX protein were more highly expressed in the L2 strain. Our results suggest that these differentially expressed transcripts may be suitable to use as molecular markers for high rates of egg production, and now need to be investigated further to assess whether they can be applied for use in breeding selection programs in Taiwanese country chickens.

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1. Introduction

An important priority in the poultry industry is to develop a rapid and accurate method to select chicken strains, which have good rates of high quality egg production. Traditional methods currently used to improve egg production efficiency and quality are very time consuming, as they assess positive genetic progress by long-term monitoring of either egg number or laying rate (Fairfull and Gowe, 1990; Atzmon et al., 2002). Since 1982, two domestic Taiwanese country chicken strains, B and L2, have been established from a common base population. There is a significantly greater egg production rate in the L2 strain (0.8) compared to the B strain (0.4), and this difference has been maintained over 20 generations (Chao and Lee, 2001). Using these two strains of chicken, we investigated whether we could identify molecular markers associated with one or other chicken strain, which could then provide the basis of a rapid selection procedure based on cDNA microarray technology.

cDNA microarrays were developed in the mid-1990s and permit the simultaneous analysis of thousands of genes (Scheda et al., 1995). The majority of microarray experiments have been conducted with human and rodent material rather than livestock species (Davoli et al., 2002; van Hemert et al., 2003; Yao et al., 2004), and although there is extensive tissue-specific gene sequence data from chickens, the field of chicken functional genomics has been relatively little explored (Liu et al., 2001; Cogburn et al., 2003). In this paper, we describe the construction of tissue-specific cDNA libraries and the genomic analysis of expressed sequence tags (ESTs) detected in shell glands isolated from B and L2 breeds of Taiwanese country chickens. The chicken shell gland is regulated by estrogen and is the site of chicken eggshell formation and accumulation, whose quality, thickness and strength is determined by the action of ion transport binding proteins (Berg et al., 2004; Vetter and O'Grady, 2005). We reasoned that this tissue is a likely source of differentially expressed genes associated with either low or high rates of egg production. We coupled cDNA library construction with cDNA microarray technology to compare global gene expression in shell glands from either the low (B) or high (L2) strains of Taiwanese country chickens during their egg-laying period. Then, we characterized the differentially expressed genes between the B and L2 strains in more detail to identify candidate molecular markers correlated with either low or high rates of egg production. We propose that the molecular markers we identify could be used to achieve early and efficient breed selection during the egg-laying stage in Taiwanese country chickens.

2. Materials and methods

2.1. Experimental animals

Low (B) and high (L2) egg production Taiwanese country chickens were bi-directionally selected over 20 generations from a common base population (Chao and Lee, 2001). The egg production rates (mean $\pm$ S.E.) of the B and L2 strain were 0.45 ± 0.11 and 0.88 ± 0.07 , respectively. Twenty-four chickens (10 of strain B and 14 of strain L2) were sacrificed 2 h post-egg-laying when they were 56 weeks of age, and the shell gland epithelium was collected in *RNAlater* (Ambion®, USA) tissue storage buffer. Total RNA was isolated from all 24 shell gland samples. Four mRNA samples purified from the L2 strain were used for cDNA library construction. Total RNA was isolated using *RareRNA* reagents (GenePure, USA), according to the manufacturer's instructions. Five samples of total RNA from each strain were used for cDNA microarray analysis and the remaining total RNA samples were used for semi-quantitative RT-PCR assays.

2.2. Chicken shell gland cDNA library construction

Messenger RNA (mRNA) was isolated from the epithelium of four L2 strain chicken shell glands using a mRNA Purification Kit (Ambion®, USA). Quantity and quality of the isolated mRNA was measured by UV/VIS spectrophotometer S2100 (WPA, Biochrom Ltd., UK) and visualized using 1% native agarose gel electrophoresis. Five micrograms of the pooled mRNA was used for cDNA library construction using the SuperScript™ Plasmid System (Invitrogen™, USA). Size fractionated cDNA was directionally ligated into the pCMV-SPORT6 vector (Invitrogen™, USA), which had been predigested with *SalI/NotI*, and then incubated at 4 °C overnight. Purified ligated products were transformed into ElectroMax DH10B competent cells (Invitrogen™, USA) by electroporation (Gene Pluser™, Bio-Rad, USA). The shell gland cDNA library was cultured overnight on LB/ampicillin agar plates at 37 °C for titration purposes, and then mixed with sterilized glycerol for long-term storage at –70 °C.

2.3. Sequence analysis

All colonies were picked from the plated library and grown in 96-well plate containing LB medium and 50 μ g/ml ampicillin. Plasmid DNA was isolated from overnight cultures using the Montage™ Plasmid Miniprep₉₆ Kit (Millipore, USA). Sequencing reactions were carried out using the ABI Prism® BigDye Terminator Cycle Sequencing Kit Version 3.1 and analyzed on an ABI 3100 Genetic Analyzer (Applied Biosystems, USA). Sequencing data were uploaded to the Phrap package software (<http://www.phrap.org/>) for quality assessment and vector sequence trimming (Ewing et al., 1998). Sequences were aligned using the basic local alignment search tool (BLAST) and the final sequences searched against the GenBank database using the BLASTN program (<http://www.ncbi.nlm.gov/BLAST/>). An *E*-value of 10^{-20} was used as a threshold for identification of gene identity.

2.4. cDNA microarray construction

A total of 2743 non-redundant transcripts were selected for cDNA microarray construction. The cDNA inserts from the isolated plasmid DNA were amplified by PCR using the SP6 (5'-ATT TAG GTG ACA CTA TAG-3') and T7 (5'-TAA TAC GAC TCA CTA TAG GG-3') primers and purified by isopropanol precipitation and washed with 70% ethanol. Final purified amplicons were

resuspended in 50% DMSO (Hegde et al., 2000) and dotted onto 384-well microplate (Corning, USA) for low density microarray construction. Clones were spotted onto the GAPS-II glass slide (Corning, USA) using the OmniGrid AccentTM microarrayer (GeneMachine, USA) loaded with eight printing pins (ArrayIt®, TeleChem, International, Inc., USA). These “in-house” cDNA microarrays were designed with each cDNA amplicon spotted in triplicate, with 210 (14 × 15) spots comprising each subarray. In addition, the housekeeping genes GAPDH, β -actin and 18S rRNA were included as positive controls to assess differences in cDNA loading differences and potential dye effects (Suchyta et al., 2004). Finally each microarray, now comprising 8238 spots in total, were cross-linked with UV at 1200 mJ/cm² and stored in a dry incubator until hybridization.

2.5. Labeling of cDNA targets and microarray hybridization

Forty micrograms of total RNA prepared from shell glands of either the B or L2 chicken strain were reverse transcribed in the presence of Cy3-dUTP (MicroMax, Perkin-Elmer, USA) to create directly labeled cDNA targets. Unincorporated fluorescent nucleotide was removed by isopropanol precipitation followed by rinsing in 70% ethanol. Purified labeled cDNA targets from each chicken strain were resuspended in 30 μ l of Hybridization buffer (MicroMax, Perkin-Elmer, USA), and hybridized to our “in-house” cDNA microarrays and protected under coverslips in humidity chambers at 65 °C overnight. cDNA targets derived from each strain were hybridized with microarrays, with five slides hybridized with the B labeled cDNA targets, and five others with the L2 derived cDNA targets, giving 10 microarray slides in total. After hybridization, the microarrays were washed by gentle agitation with 0.5 × SSC/0.1% SDS, 0.06 × SSC/0.1% SDS and 0.06 × SSC for 15 min each at room temperature. Slides were dried by centrifugation in 50 ml conical tubes at 2000 rpm for 2 min. The microarray slides were scanned at 532 nm (for Cy3) using the GenePix 4000B microarray scanner (Axon Instruments, Union City, CA, USA), and then the GenePix Pro 4.1 software was used to acquire the array images, align the spots, quantify their intensity and finally to export the GPR files containing spot intensity raw data.

2.6. Microarray data analysis

The 10 microarrays, five sets from each strain, were scanned and data quantified using the GenePix 4000B scanner and GenePix Pro 4.1 software (Axon Instruments, Union City, CA, USA). Spot intensities were calculated, using the median value of all spots for further analysis. Poor-quality spots (flag $\neq$ 0) were filtered from the raw data of each array before analysis, followed by background subtraction of median intensity signals (F_{532} median – B_{532} median) and then rescaled by global normalization (Quantile) using “Avadis” software (Strand Life Sciences, USA). Net intensities less than 500 from the normalized data were removed and the remaining signals transformed logarithmically. Statistically significant differential transcript expression was characterized from five sets of microarray data derived from each strain, using the unpaired *t*-test. In order to select significant gene expression changes, transcripts showing statistically different ($P < 0.05$) expression levels, representing three-fold or greater were selected and processed for further analysis by semi-quantitative RT-PCR to validate the result.

2.7. Semi-quantitative RT-PCR analysis

Semi-quantitative RT-PCR was used to confirm the expression levels of differentially expressed transcripts derived from our cDNA microarray data. Two micrograms of total RNA extracted

Table 1
The designed specific-primers were used for semi-quantitative RT-PCR

Name	Primer	Sequences (5' → 3')	Annealing (°C)
OCX-32	F	AGAAGAGGACCACAGATTTTACGAA	56
	R	GATGGTCATGGGCAATATTTCC	
EEF2	F	TCATAGCGATTGGAGACTTTCAAC	55
	R	CATGAGTCATGTGCAACACAGAA	
ANXA2	F	GCATATGCCACAGTTAAGGCTTAC	56
	R	TCATTGCTGCGGTTTGTC	
SOX18	F	CTGGATGCTCCTTTAGATGAATGTC	56
	R	CCATCACCCATATTTTCATCACA	
COTL1	F	CCTGCCGTGCTCTATCTCATG	55
	R	CAGGACCTTGCATCAAAATGAC	
MX	F	TCAAGAGTGGTCGGTGTGCGATA	58
	R	TTGGACCATGGATTGTTTCATGT	
18S rRNA	F	CTCCCCTCCCGTTACTTGGA	58
	R	TTGGTTTTGGTCTGATAAATGCA	

F: forward; R: reverse.

from individuals of each strain ($n=5$ chickens/strain) were reverse transcribed to create first strand cDNA using the MasterAmpTM High Fidelity RT-PCR Kit (Epicentre[®] Biotechnologies, USA). Specific primers were designed using the Primer Express Version 2.0 software (Applied Biosystems, USA) and the cycling programs used are listed in Table 1. PCR was carried out in a final volume of 25 μ l containing 10 $\times$ PCR buffer, 2.5 mM dNTPs, 10 μ M of each primer, 20 ng of template cDNA and 0.5 U of *Taq*-polymerase (DyNAzyme II DNA Polymerase, Finnzymes, Finland). PCR products were separated on a 2% agarose gel and visualized by staining with ethidium bromide. The band densities were measured with Analyze A 1D Image software (TotalLab, Ultra-Lum, USA). The 18S rRNA was used as an internal control for calibration purposes.

3. Results

3.1. Sequence analysis of the chicken shell gland cDNA library

A total of 7680 colonies were randomly selected from our L2 chicken shell gland cDNA library and processed for 5'-end sequence analysis. Inadequate sequences were removed before quality assessment and vector sequence trimming of the remainder, which were then searched against the GenBank database using the BLASTN program. Cluster analysis of 5286 sequenced clones revealed that 2445 non-redundant sequences were matched to known genes based on BLASTN matches with an E -value $<10^{-20}$; the other 603 non-redundant sequences with an E -value $>10^{-20}$ were classified as "Unmatched" which suggested our sample may contain some novel genes (Hatey et al., 1998). Of these 2445 non-redundant sequences, 1639 (54%) were homologous to *Gallus gallus* genes, 501 (16%) were *Homo sapiens* genes and the remaining 305 (10%) genes showed homology with other species. All 2743 non-redundant transcripts, comprising the 2140 transcripts "matched with *G. gallus* and *H. sapiens*" and 603 "unmatched" transcripts were used to construct our cDNA microarrays.

3.2. cDNA microarray analysis

Each shell gland cDNA microarray contained a total of 8238 spots, comprising the 2743 non-redundant transcripts, and three internal control cDNAs of GAPDH, β -actin and 18S rRNA. All elements were spotted in triplicate to improve consistency and statistical analysis within an individual microarray. To confirm reproducibility and variability across each array, we calculated the correlation coefficients for all 10 hybridization results. High correlation coefficients ($R^2 = 0.9639$) were obtained from two arrays hybridized with the B chicken strain (Fig. 1A), and similar results were obtained using cDNA targets from the L2 chicken strain. The mean signal-to-noise ratio (mean $\pm$ S.E.) (Wang et al., 2001) from our “in-house” cDNA microarrays was 0.80 ± 0.01 . This result confirms that our cDNA microarray hybridization data is highly reproducible and reliable.

We conducted each experiment in five replicates so that there were, in total, 10 sets of microarray hybridization data. Five arrays used cDNA targets derived from five different samples taken from the L2 strain, and the other five sets used cDNA targets extracted from B strain chickens. One of the microarray transcript expression profiles obtained after hybridization with B and L2 strain cDNA targets is shown in Fig. 1B. This reveals that the majority of the hybridization spots have the same signal intensity. We used the statistics analysis tool “Avadis” to search for differentially expressed transcripts from the 10 arrays and Fig. 1C shows the log intensity of 558 differentially expressed transcripts ($P < 0.05$), using a scatter plots to compare the B strain ($n = 5$) versus the L2 strain ($n = 5$). We imposed a cutoff so that only those transcripts that showed a ≥ 3 -fold change in expression between shell glands from B and L2 strain chickens were analyzed further; in all a total of 85 transcripts (Fig. 1C). Functional annotation revealed that 51 transcripts were ESTs (data not shown) and 34 transcripts were involved in signal transduction, protein biosynthesis, cellular metabolism, cell adhesion, cell organization and biogenesis (Table 2). Sixteen transcripts showed higher expression in chicken shell glands from the B strain compared to the L2 strain and 18 transcripts were expressed at higher levels in the L2 strain compared to the B strain. One transcript, eukaryotic elongation factor 2 (EEF2), was 12-fold elevated in the B strain relative to the L2 strain (Table 2). There were three transcripts with unknown function, and five other transcripts involved in excretion, stress response, transport, muscle contraction and the immune response.

3.3. Semi-quantitative RT-PCR analysis of differentially expressed transcripts

We used semi-quantitative RT-PCR to confirm that the differential transcript expression detected by our microarrays was consistent with transcript levels present in B and L2 chicken shell gland tissue ($n = 5$ chicken/strain). As predicted from our microarray data, we showed that B strain chickens expressed more transcripts of the eukaryotic elongation factor 2 (EEF2), ovocalyxin-32 (OCX-32) and annexin A2 (ANXA2) compared to L2 hens at egg-laying stages. Higher levels of coactosin-like 1 (COTL1), transcription factor SOX18 and MX protein were detected in the shell glands of L2 compared to the B strain, again in keeping with our microarray data. In total, six selected differentially expressed transcripts, originally detected in our microarrays, showed the same pattern of expression when analyzed by semi-quantitative RT-PCR (Fig. 2). This positive correlation suggests that our microarray data is an accurate reflection of levels of transcript expression present in the eggshell gland tissue taken from the two chicken strains.

4. Discussion

We describe the creation of a chicken shell gland cDNA library, and the construction of a cDNA microarray, which was then hybridized with cDNA targets derived from two chicken strains, B

Table 2

List of differentially expressed transcripts in shell glands from B and L2 strains of Taiwanese country chickens

Accession no.	Description	Symbol	Species	B/L2 ^a	Biological process
EC911969	Growth factor receptor-bound protein 10	GRB10	<i>Homo sapiens</i>	4.28	Signal transduction
EC911970	AT-rich interactive domain 4B	ARID4B	<i>H. sapiens</i>	3.80	Unknown
EC911971	HtrA serine peptidase 1	HTRA1	<i>H. sapiens</i>	3.72	Cellular metabolism/signal transduction
EC911972	Intraflagellar transport 88 homolog	IFT88	<i>H. sapiens</i>	3.35	Excretion
EC911973	Eukaryotic translation initiation factor 2C, 3	EIF2C3	<i>H. sapiens</i>	3.29	Protein biosynthesis
EC911974	Eukaryotic translation elongation factor 2	EEF2	<i>Gallus gallus</i>	12.02	Protein biosynthesis
EC911975	Putative alpha3-fucosyltransferase	FUT10	<i>G. gallus</i>	8.69	Cellular metabolism
EC911976	Ovocalyxin-32	OCX-32	<i>G. gallus</i>	6.13	Cellular proliferation/skeletal development
EC911977	Annexin A2	ANXA2	<i>G. gallus</i>	5.22	Skeletal development
EC911978	Vinculin	VCL	<i>G. gallus</i>	3.63	Cell adhesion
EC911979	CCCTC-binding factor (zinc finger protein)	CTCF	<i>G. gallus</i>	3.61	Cellular metabolism
EC911980	G-protein-coupled receptor	GPR34	<i>G. gallus</i>	3.28	Signal transduction
EC911981	Heat shock 70 kDa protein 5	HSPA5	<i>G. gallus</i>	3.21	Stress response
EC911982	v-ets erythroblastosis virus E26 oncogene like	ERG	<i>G. gallus</i>	3.06	Cellular metabolism
EC911983	Decorin	DCN	<i>G. gallus</i>	3.04	Organization and biogenesis
EC911984	Frizzled-related protein	FRZB	<i>G. gallus</i>	3.03	Signal transduction/skeletal development
EC911985	Catenin (cadherin associated protein), alpha 1	CTNNA1	<i>H. sapiens</i>	−6.64	Cell adhesion/organization and biogenesis
EC911986	Preimplantation protein 3	PREI3	<i>H. sapiens</i>	−5.50	Transport
EC911987	Ornithine decarboxylase antizyme 2	OAZ2	<i>H. sapiens</i>	−4.57	Cellular metabolism
EC911988	X box-binding protein spliced form	XBP1	<i>H. sapiens</i>	−3.30	Cellular metabolism
EC911989	Mitochondrial tumor suppressor gene 1	MTSG1	<i>H. sapiens</i>	−3.07	Signal transduction
EC911990	Cleavage and polyadenylation specific factor 6	CPSF6	<i>H. sapiens</i>	−3.05	Cellular metabolism
EC911991	Coactosin-like 1 (dictyostelium)	COTL1	<i>H. sapiens</i>	−3.03	Unknown
EC911992	L-caldesmon	CALD1	<i>G. gallus</i>	−5.48	Muscle contraction
EC911993	Cryptochrome 1 (photolyase-like)	CRY1	<i>G. gallus</i>	−4.73	Cellular metabolism/circadian rhythm
EC911994	Zyxin	ZYX	<i>G. gallus</i>	−4.48	Cell adhesion/signal transduction
EC911995	Hydroxymethylglutaryl-CoA lyase	HMGCL	<i>G. gallus</i>	−4.17	Cellular metabolism
EC911996	FK506 binding protein 3	FKBP3	<i>G. gallus</i>	−3.87	Cellular metabolism
EC911997	Transcription factor SOX18	SOX18	<i>G. gallus</i>	−3.71	Cellular metabolism
EC911998	WD repeat and SOCS box containing 1	WSB1	<i>G. gallus</i>	−3.71	Unknown
EC911999	ras-related C3 botulinum toxin substrate 3	RAC3	<i>G. gallus</i>	−3.44	Organization and biogenesis
EC912000	Mx protein	MX	<i>G. gallus</i>	−3.40	Immune response
EC912001	Aldehyde dehydrogenase-6	ALDH6	<i>G. gallus</i>	−3.33	Cellular metabolism
EC912002	Enolase 1, (alpha)	ENO1	<i>G. gallus</i>	−3.21	Cellular metabolism

^a B/L2: the log₂ ratio of transcripts expression level of B to L2 strains.

Fig. 1. (A) Logarithmic scatter plot of the fluorescent intensities of hybridized cDNA microarray clones. The high correlation coefficient shown here confirms that our cDNA microarrays were constructed in a reproducible and reliable manner. (B) cDNA microarray analysis of differentially expressed transcripts in shell glands from strains B and L2 Taiwanese country chickens. The digital image of the hybridization results using one of five microarrays derived from each strain is illustrated. (C) Scatter plots of fluorescent intensity from 10 normalized cDNA microarray data. Each strain represents the mean value of five microarray data. There were 85 differential expressed transcripts ($P < 0.05$), representing three-fold or greater using the “Avadis” software.

and L2. This differential transcript analysis method has allowed us to identify potential molecular markers associated with different rates of egg production ratio in two chicken strains derived from the same genetic background of Taiwanese country chickens (Chao and Lee, 2001; Kuo et al., 2005). The BLAST analysis of the non-redundant transcripts we detected identified a high percentage of “unmatched” sequences (20%) due to the high *E*-value that we employed in our

Fig. 2. Confirmation of differentially expressed gene expression by semi-quantitative RT-PCR (semi-q RT-PCR). Differential expression of transcripts expressed in shell glands from B ($n=5$) and L2 ($n=5$) strains of Taiwanese country chickens using “in-house” cDNA microarrays. The red open box indicates that the mRNA levels of OCX-32, EEF2 and ANXA2 are more highly expressed in the B strain, whereas expression of the SOX18, COTL1 and MX transcripts are more highly expressed in the L2 strain. Relative values of the expression of the indicated transcripts were confirmed by semi-q RT-PCR. The mean ratio of each detected transcript was derived from five independent samples, then using one sample to represent a whole strain.

analysis. Further, the high correlation coefficients we achieved between our microarray replicates suggests that the method we used to construct our arrays and carry out microarray hybridization is reliable and highly reproducible (Fig. 1A).

Using microarray hybridization methods we isolated a number of differentially expressed transcripts (Fig. 1C). Most of the transcripts we identified were related to signal transduction, protein biosynthesis, cellular metabolism, cell adhesion, cell organization and biogenesis (Table 2), as defined by the Gene Ontology (Al-Shahrour et al., 2004) (<http://www.fatigo.org>). In addition, other transcripts were associated with excretion, the stress response, transport, muscle contraction and the immune response. Six differentially expressed transcripts were selected from our microarray data and analyzed using semi-quantitative RT-PCR to confirm that they were also expressed at different levels in shell gland tissue (Fig. 2). In addition four additional transcripts isolated from chicken shell glands were investigated to define their known and potential interactions in chicken shell glands from low (B) and high (L2) egg production strains.

Two of these four interesting transcripts, ovocalyxin-32 (OCX-32) and annexin A2 (ANXA2) were expressed at 6.13- and 5.22-fold higher levels, respectively, in the shell glands from strain B, compared to the L2 strain (Table 2). OCX-32 is a novel matrix protein synthesized in the distal oviduct and secreted by surface epithelial cells as eggshell formation takes place (Gautron et al., 2001). The timing of OCX-32 secretion into the chicken uterine fluid suggests that it may play a role in the process of mineral deposition and completion of the eggshell (Hincke et al., 2003). We found the higher percentage of eggshell in eggs from B strain chickens compared to the L2 strain (data not shown) matched the results we obtained from our semi-quantitative RT-PCR analysis, which confirmed that OCX-32 expression levels were higher in B chickens (Fig. 2). The differentially expressed transcript, annexin A2 (ANXA2), we isolated is a Ca^{2+} binding

protein related to the cytoskeleton proteins which bind to F-actin and phospholipids in a calcium dependent manner (Chailley and Pradel, 1992). It may interact with OCX-32 during the process of mineral deposition and we showed that the semi-quantitative RT-PCR result we obtained with this transcript was identical with our microarray data (Fig. 2). Therefore, we suggest that OCX-32 and ANXA2 are potential molecular markers associated with different rates of egg production, and so can be used for breed selection in Taiwanese country chickens.

Two other interesting transcripts, MX protein and transcription factor SOX18 were expressed at 3.40- and 3.71-fold higher levels, respectively, in chicken shell glands from L2 chickens compared to the B strain (Table 2). MX protein was first identified in laboratory mice, is induced by interferon, and contains putative tripartite GTP-binding sites, which inhibit viral infection and replication (Horisberger and Gunst, 1991; Ko et al., 2002; Lin et al., 2005). Reports initially suggested that MX proteins do not have antiviral activity in chickens (Bernasconi et al., 1995); however, later experiments indicated that the GTP-binding domains and leucine repeats were highly conserved among MX proteins, and a *Ser* to *Asn* substitution close to the carboxy terminus determined actual antiviral activity in chickens (Ko et al., 2002, 2004). In mammals, MX proteins are proposed to act as a pregnancy recognition signal and, in keeping with this role, MX transcripts are up-regulated in uterine tissue during the estrous cycle and early pregnancy (Ott et al., 1998; Hicks et al., 2003). The transcription factor SOX18 belongs to the SOX (*Sry*-related HMG box) gene family that controls cell growth and differentiation in the presence of high concentrations of estrogen 17 β -estradiol (E2) in the uterus (Moggs et al., 2004). A higher concentration of E2 was detected in L2 strain compared with the B strain (data not shown) which suggests that the SOX18 induced by E2 may play an important role in egg production. As yet, there is no direct evidence that MX and SOX18 proteins interact, however, it is possible that they are both associated with the high rates of egg production observed in the L2 strain of Taiwanese country chickens.

The chicken genome project was completed in 2004; however, the functional gene database of *G. gallus* remains incomplete. This means that the molecular interactions between chicken proteins are not fully defined, so the gene ontology of the candidates we identified were explored using information from *H. sapiens*, *Mus musculus*, *Rattus norvegicus* and other species using the FatiGO classification (Al-Shahrour et al., 2004). Microarray data is an important resource from which to infer gene networks and to identify highly conserved genetic pathways operating in specific livestock or poultry species (de la Fuente et al., 2002; Stuart et al., 2003).

In conclusion, we have generated a chicken shell gland cDNA library from the L2 strain of Taiwanese country chickens, and have used “in-house” cDNA microarray assays to screen out differentially expressed transcripts between low (B) and high (L2) egg production strains. A number of interesting transcripts were selected for semi-quantitative RT-PCR, which confirmed our microarray results. We propose that the differentially expressed transcripts identified here may serve as potential molecular markers associated with high rates of egg production, and so provide the basis of rapid and early breed selection method, operating prior to the egg-laying stage in Taiwanese country chickens.

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