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The chicken genome and the developmental biologist

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Review

The chicken genome and the developmental biologist

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Abstract

Recently the initial draft sequence of the chicken genome was released. The reasons for sequencing the chicken were to boost research and applications in agriculture and medicine, through its use as a model of vertebrate development. In addition, the sequence of the chicken would provide an important anchor species in the phylogenetic study of genome evolution. The chicken genome project has its roots in a decade of map building by genetic and physical mapping methods. Chicken genetic markers for map building have generally depended on labour intensive screening procedures. In recent years this has all changed with the availability of over 450,000 EST sequences, a draft sequence of the entire chicken genome and a map of over 1 million SNPs. Clearly, the future for the chicken genome and developmental biology is an exciting one. Through the integration of these resources, it will be possible to solve challenging scientific questions exploiting the power of a chicken model. In this paper we review progress in chicken genomics and discuss how the new tools and information on the chicken genome can help the developmental biologists now and in the future.
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1. Why sequence the chicken genome?

The main arguments to sequence the chicken genome were to benefit agriculture (poultry breeding and animal health) and medicine (human health and a model of vertebrate development), and to provide a key anchor species in which to understand the evolution of vertebrates. As one of the most important sources of protein in the world the arguments to find out more about the chicken for agriculture are obvious. Chicken embryos are one of the main vertebrate models of development studied in biology and medicine—a fact known to all readers of *Mechanisms of Development*. Its key advantage is that experimental manipulations can be carried out in vivo, while the embryo is still in the egg. Classical manipulations have included ablation and transplantation of tissues within chick embryos to study cell–cell interactions, cell determination and patterning. A wide range of other manipulations is now possible, including the construction of chick/quail chimeras to study cell fate, the grafting of microcarrier beads releasing defined molecules, such as growth factors, and genetic manipulations. Gene constructs can be readily introduced into chicken embryos using retroviral methods or electroporation. These techniques have been widely used to over-express genes at particular times and at specific locations within the developing chicken embryo. Recent reports suggest that *RNAi* should also be readily applicable in chicken embryos (Ui-Tei et al., 2003; Pekarik et al., 2003). This highlights the possibility of using the chick embryo as a high-throughput tool for testing vertebrate gene function (reviewed by Brown et al., 2003). The origins of mammals and birds can be traced back to a common ancestral species, 300–350 million years ago. Thus the study of the chicken genome will be as relevant to the evolution of mammals as it is to other birds. This may be at the level of the genes and proteins, to the organisation and regulation of genes, and the evolution of regulatory pathways used in the development of vertebrates. Early in March 2004 the first draft sequence of the chicken genome was announced (see below for more details). The aim of this review is to discuss these developments and how they will impact on developmental biology.

2. The chicken karyotype

The chicken genome has a haploid content of 1.2×10^9 base pairs of DNA and is divided among 39 chromosomes

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including nine pairs of cytologically distinct macrochromosomes and 30 microchromosomes (Burt, 2002). The sex chromosomes are denominated Z and W; in birds, unlike mammals, it is the males that are homogametic (Z/Z), while the females are heterogametic (Z/W). The 30 chicken microchromosomes contain about one-third of the genomic DNA but until recently, these chromosomes were thought to be inert. A number of recent studies have now shown that the microchromosomes are in fact gene-rich, with recent estimates suggesting that microchromosomes contain at least as twice as many genes as the macrochromosomes (McQueen et al., 1996; Smith et al., 2000).

3. Genome maps

Maps of the chicken genome come in many types and levels of resolution and are based either on genetic linkage or physical mapping techniques, including FISH (fluorescence in situ hybridisation), RH (radiation hybrid) mapping, contig building from genomic clones or the genome sequence itself. These maps have been used in genetic linkage studies, creating the first comparative maps between the chicken and other vertebrates, and have been used to anchor the genome sequence to chromosomes and provide additional support for the sequence assembly. These maps can be viewed as the organising structure from which to access the information within the chicken genome.

Recent developments in the isolation of genomic clones (mostly in cosmid and bacterial artificial chromosome or BAC cloning vectors) and individual microchromosomes by microdissection have created a universal set of DNA probes or so-called 'landmark probes' specific for each chromosome (Masabanda et al., 2004). It is now relatively simple to map any cloned gene to a specific chromosome; even a microchromosome using two-colour FISH using these probes (Fillon et al., 2003). The first genetic linkage map for any livestock species was the chicken (Hutt, 1936) and so it is fitting that it is also the first livestock species to be sequenced. The development of the current genetic linkage map can be traced back to the use of a few reference-mapping families in the mid-1990's (Levin et al., 1994). A wide range of genetic markers were used in the early stages of this map, including: RFLP's or restriction fragment length polymorphism's, RAPD or randomly amplified polymorphic DNA's, CR1-repreats and classical phenotypic markers, such as sex-linked dwarfism. The driving force for developing a map of genetic markers was the desire to perform whole genome linkage to map quantitative trait loci (QTL) that control quantitative traits. Therefore there was a shift to mapping more markers suited to high-throughput methods, such as those based on microsatellite sequences and AFLP's or amplified-length-fragment-polymorphism's (Schmid et al., 2001). From an analysis of markers used across several reference-mapping populations a consensus map of over 2000 loci, spanning 4000 cM was constructed

and has served as the standard genetic map (Groenen and Crooijmans, 2003).

A major limitation of genetic markers for the construction of gene maps has been the need to identify polymorphisms, necessary to track their inheritance in linkage studies. The use of RH mapping panels to construct gene maps of many other species has increased the rate of gene mapping significantly. In this method, the presence or absence of a marker is only required (usually based on a PCR assay) and there is no need to identify polymorphisms. Recently a chicken RH panel was constructed (Morisson et al., 2002) and early results for chromosomes 7 and 15 have produced RH maps, which are co-linear with the genetic map (Morisson et al., 2003; Jennen et al., 2004). The use of this RH panel will complement other physical mapping efforts and the assembly of the chicken genome sequence.

Large insert genomic libraries based on BAC clones have been used to create a physical map of the entire chicken genome based on overlapping clones (Ren et al., 2003). BAC libraries have been constructed from a White Leghorn line (Crooijmans et al., 2000) and an inbred Jungle Fowl line (Lee et al., 2003). The latter was used in the sequencing project (see below). The Washington University Genome Sequencing Centre (http://genome.wustl.edu/projects/chicken/) has fingerprinted over 188,000 BAC clones from many of these libraries and has constructed a BAC physical map of 260 contigs based on over 143,000 BAC fingerprints. Over 75% (202/260 contigs) of the BAC contigs have been anchored to a chromosome mostly by the work of Romanov et al. (2003) using an oligonucleotide hybridisation strategy. The development of the chicken BAC map has been an important step in the assembly of the chicken genome sequence (Aerts et al., 2003).

4. Chicken genome sequencing

In 2003 the National Human Genome Research Institute funded a project to sequence the chicken genome at the Washington University Genome Centre (WUGSC) (Burt and Pourquié, 2003). In March 2004, a 6.6-fold assembly of the chicken genome was completed and can be accessed from a number of genome browsers (for a complete list see http://www.chicken-genome.org/). The DNA of a single female of the UCD001 inbred Red Jungle Fowl line (Crittenden et al., 1993) was sequenced. Sequencing was mostly based on the whole genome shotgun approach, supplemented with sequences from fosmid and BAC-ends. The parallel computation assembly program or PCAP (Huang et al., 2003) was used for sequence assembly. In brief, PCAP identifies and removes repetitive regions, contaminated end regions and chimeric reads. Then assembles individual sequence reads into contigs based on unique overlaps. These contigs are then linked into scaffolds based on sequence information from each end of each of

the genomic sub-clones (or 'paired-ends'). In total over 11 million sequence reads were collected with genome coverage of 6.6-fold (Wes Warren, personal communication). The PCAP assembly produced 98,612 contigs over 1 kb in length. The average contig length was 11 kb (maximum length 442 kb). Further assembly produced a scaffold of 32,767 supercontigs with an average length of 32 kb (maximum length of 33.5 Mb). Further assembly of clone and sequence information was made possible by anchoring supercontigs to the BAC map of the chicken genome. Final improvements were made using information from genetic and physical mapping data (LaDeana Hillier, personal communication). Comparisons with EST sequences, complete mRNA sequences and 6 Mb of fully sequenced regions suggests that more than 90% of the genome has been sequenced. Extensive analysis of the chicken genome sequence is underway and the first phase will be complete June 2004. Initial results can be viewed from all the major genome browsers (Fig. 1).

The identification of genetic variation in the chicken genome, for example, between broiler and layer lines of chickens, will be an important step in understanding the genetic basis of breed differences. Single nucleotide polymorphisms or SNPs are the most frequent type of polymorphism in a vertebrate genome (Ben-Ari et al., 2004). The first attempts to build SNP maps in the chicken were based on the analysis of EST sequence data derived from multiple animals and strains (expressed sequence tags, see Section 5 below for more details on EST programmes). The University of Delaware chicken SNPs homepage (http://chicksnps.afs.udel.edu/) contains a searchable database of these chicken cSNPsn (Emara and Kim, 2003). At UMIST a set of over 11,000 high quality SNPs were extracted from 350,000 ESTs and are searchable via the chickEST www site (http://www.chick.umist.ac.uk/). Recently collaboration between the Beijing Genome Institute, The Wellcome Trust (UK), University of Uppsala and the Roslin Institute used a whole genome shotgun approach to catalogue genetic variation between multiple chicken lines, including broiler, layer, Silkie and the Red Jungle Fowl (the strain used in the genome sequence at WUGSC). Initial analysis of these results has detected about two million putative SNPs distributed at a frequency of 1 per 250 base pairs. The results can be viewed from the Chicken Genome Browser Gateway at UCSC (http://genome-test.cse.ucsc.edu/cgi-bin/hgGateway?db = galGal2) and more details will be available in June 2004 (Gane Ka-Shu Wong, personal communication).

numbers of randomly selected cDNA clones isolated from libraries constructed from a range of tissues. Ideally cDNA libraries should be normalised to reduce the redundancy in sequencing ESTs. These sequences are clustered using bioinformatics software into unique clusters, each representing a putative gene. Currently there are 460,577 chicken ESTs in the latest release of dbEST (March 2004, version 031904). Major EST programmes in the chicken include those carried out at the University of Delaware (Tirunagaru et al., 2000), the GSF (Abdrakhmanov et al., 2000) and the largest, by Boardman et al. (2002) characterised over 340,000 ESTs from libraries taken from 21 different adult and embryonic tissues. Annotation of the EST data (Boardman et al., 2002; Brown et al., 2003) revealed that about 40% of the clustered sequences have orthologs in other species represented in the current sequence databases. This chicken gene catalogue is therefore likely to contain genes either specific to the chicken (and birds) or not yet characterised in other species. A joint project between UMIST, Dundee, GSF and the Sanger Institute is sequencing over 10,000 putative full-length cDNAs from these EST collections. Information on these chicken ESTs has been organised in a searchable database, chickEST (http://www.chick.umist.ac.uk/). For developmental biologists interested in specific genes, it is possible to search for sequence homology using a number of BLAST programs or tissue specific patterns of expression by in silico subtraction. ChickEST provides a database of predicted tryptic peptides based on a translation of all the available EST and cDNA sequence data. These EST resources have also served as the basis of the first generic 13K chicken gene cDNA microarray; now available from facilities in the UK (Roslin Institute: http://www.ark-genomics.org/) and the USA (Fred Hutchinson Cancer Research Center: genomics@fhcrc.org). In addition, there are many custom arrays, for example, Larry Cogburn at the University of Delaware has produced two chicken microarrays: a 10K metabolic/somatic and a 8K neuroendocrine/reproductive system array. These are being used for transcriptional profiling in tissues of divergently selected broiler chickens (See http://udgenome.ags.udel.edu/~cogburn/ for more information). Finally, these EST and cDNA resources have been critical in the annotation of the genes in the chicken genome sequence and the prediction of the intron–exon structures of genes (Ewan Birney, personal communication). These EST resources provide exciting opportunities for gene expression studies and proteomics and devising new tests for gene function using chick embryos.

5. ESTs and full-length cDNA clones

A powerful tool for gene discovery has been the isolation and characterisation of expressed sequenced tags or ESTs. In this method the partial sequences (about 500-bp from either the $5'$- or $3'$-end or both) are determined for large

6. Evolution and comparative genomics

The chicken shares a common ancestor with mammals about 300–350 million years ago and is placed as an important anchor species in any evolutionary study. Comparisons with the chicken are used to study

the evolution of specific genes or gene families or genome organisation itself—these are the structural views. Phylogenetic studies can also be used to examine the evolution of developmental mechanisms and signalling pathways—the functional views.

Before the chicken genome sequencing effort, extensive sequencing of large segments in the chicken were limited, for example, the T-cell receptor beta-chain constant region (AF110982), T-cell receptor alpha chain constant region (U83833, Wang et al., 1997), alpha-globin gene cluster (AF098919, AY016020, Flint et al., 2001), beta-globin gene cluster (L17432), MHC complex (AL954802, AL023516, Kaufman et al., 1999), SCL locus (AJ131018, Göttgens et al., 2000), class II cytokine receptor gene cluster (AF082667, Reboul et al., 1999) and to specific regions in the chicken orthologous to human chromosomes 7, 11 and 14 (Green et al., www.nisc.nih.gov/open_page.html? projects/zooseq.html). The general conclusions from these early studies have been that chicken genes tend to be 2–3 times smaller than that found in mammals and that gene order is conserved over regions of at least 1 cM or 300 kb. The largest sequence is for a region orthologous to human chromosome 7q31.2. This sequence is 414 kb and covers five overlapping BAC clones. The order of the genes is identical (TES, CAV1, MET, CAPZA2 and ST7) to that found in the human genome, which covers a 1-Mb region. Another example is the 305-kb IGF2 region on chicken chromosome 5, also identical in gene order to its human counterpart (IGF2, INS, TH, ASCL2 and CD81).

Until the genome sequencing effort, the most detailed comparative maps between chicken, mouse and human were based on genetic and physical maps (Burt, 2002). Large conserved segments are found on chicken chromosomes 4–8. Closer inspection, however, reveals one or more intra-chromosomal rearrangements. A detailed comparison of human chromosome 15 with chicken orthologues (Crooijmans et al., 2001) identified seven conserved segments on chicken chromosomes 1, 5 and most on 10, based on 91 mapped orthologues. However, a high-resolution comparative map of chicken chromosome 10 and human chromosome 15 revealed 19 conserved gene orders. This result indicates that there have been at least 16 intra-chromosomal rearrangements since the divergence of human and chicken. But this result does not indicate any lineage specific rates of intra-chromosomal rearrangement—this requires an outgroup species, such as the zebrafish, as suggested by Crooijmans et al. (2001). However specific rearrangements in the mouse or human lineage were identified, using the chicken as the outgroup species. The same approach has been used by the Wageningen group to construct high-resolution comparative maps of chicken chromosomes 13 (Buitenhuis et al., 2002) and 24 (Jennen et al., 2002). Genes mapped to chicken chromosome 13 have orthologues on mouse chromosomes 11, 13 and 18, and only human chromosome 5. At least one intra-chromosomal rearrangement was detected between chicken and human. Examination of the high-resolution comparative maps between human chromosome 11 and chicken 24, however, reveals at least four intra-chromosomal rearrangements. With a gene map based on a comparison of human and chicken genome sequences it will be possible to examine the question of conservation of gene order and identify intra-chromosomal rearrangements within the microchromosomes.

7. Future prospects and possibilities

So with the sequence of the chicken genome now available what are the prospects for the future for the chicken genome and the developmental biologist?

7.1. Genes—expression and function

Integration of all available evidence (ESTs, cDNAs, homologies, etc.) with the chicken genome sequence will provide a catalogue of all chicken genes. This will include the prediction of the intron–exon structure and putative control regions. Transcription and translation of these genes will provide a catalogue of all chicken proteins. The function of these sequences will be predicted at first, mostly by comparisons made with the proteins characterised in other species. These links will go as deep as the amino acid homologies hold out at least in all vertebrates and possibly other model organisms such as Drosophila—another important model for developmental biology. However, developmental biologists will exploit this information and the new tools (see below) and the power of the chicken embryo to establish new functions for genes in development.

7.2. Genome—gene organisation and regulation

For the first time the genome sequence will provide a wider context in which to consider the chicken genes. The role of gene organisation and clusters will be examined. The role of short and long distance regulation of gene function will be under study.

7.3. Evolution—genes, families and pathways

One of the reasons for sequencing the chicken genome was its place in the evolutionary tree of vertebrates. The genome sequence will be compared at first to the genomes of human and mouse, and will uncover conserved regions. These will represent the coding and non-coding regions. It is predicted that these comparisons will confirm and predict new genes found in birds and mammals. The chicken will be an ideal outgroup for studies on the evolution of gene and protein families. In many cases we expect the chicken to have fewer genes and smaller gene families, but there will always be surprises. The conservation of signalling molecules and regulatory factors will help to understand

the evolution of developmental pathways. The difference between chickens and mammals will also help to understand our uniqueness.

7.4. New tools—bioinformatics, genes (ESTs, arrays), proteomics and RNAi

The genome sequence and all the resources that were developed within the project will be a treasure trove for the experimental biologist. Access to information on the chicken genome and its biology will require developmental biologists to be conversant in bioinformatics (see databases below). The EST and cDNA resources will be used as tools to examine gene expression (e.g. using cDNA microarrays or DNA chips). The full or nearly full-length cDNA clones can be used for expression of protein products and over expression in transient systems in the chicken embryo. The use of RNAi will complement these expression studies and provide a test of function. Genetic markers will be used to map and identify genetic mutants—provided the mutant strains are maintained.

### 7.5. The chicken genome and increased quality

The availability of a draft sequence of the chicken genome is amazing and will provide a great boost to chicken biology. However, it is only a draft sequence and is likely to cover 90% of the genome with an average error rate of less than 1%. So in the coming months and years it will be essential to complete the sequence at least to 99% coverage, so we have all genes assembled and identified.

7.6. Gene expression patterns—an atlas, ontology and a repository of images

The pattern of gene expression as determined by in situ hybridisation has been a revolution in developmental biology. The new tools and information on the chicken genome are only going to make this richer. However, there is going to be a need for some standards to improve communication and the exchange of results. There is going to be a need for an electronic atlas of chick embryology and anatomy, and an agreed ontology of terms to describe what we see (Davidson and Baldock, 2002). This information can
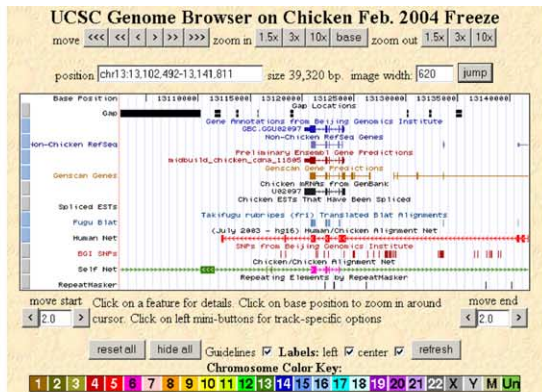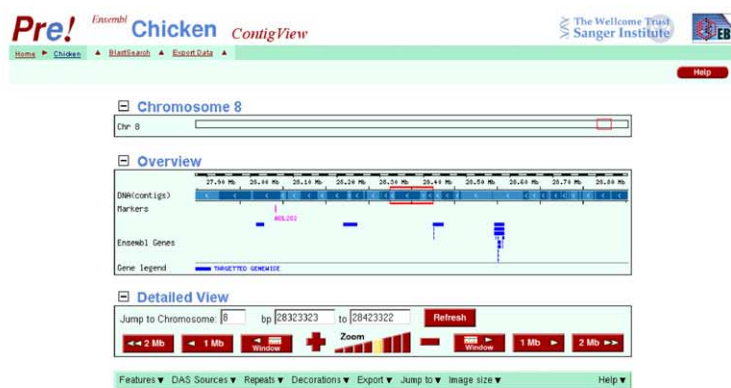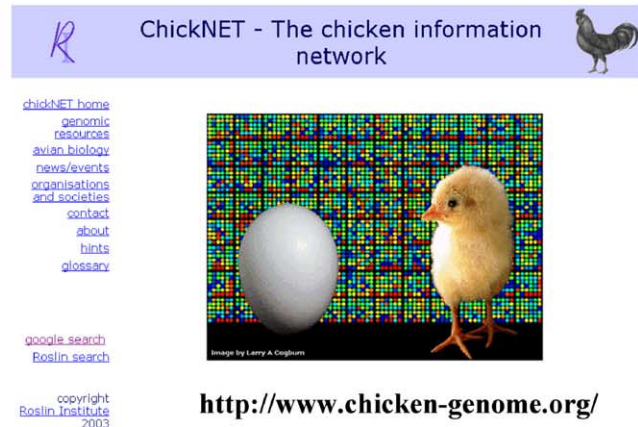

Fig. 1. Access to information on the chicken: genome browsers and WWW sites.

then be used to annotate images of gene expression patterns either in sections or whole-mount in situ hybridisations. This will require access to stored images and links to the genome sequence.

7.7. Pathways—protein–protein interactions, pathways and tertiary structure

There is much talk about proteomics, but the key interests in this area will be in interacting systems. The signalling pathways as determined by gene expression patterns and protein–protein interactions (from yeast 2-hybrid and mass spectrometry analyses). Finally the structure of proteins from other organisms will be of interest to model chicken proteins and uncover patterns of gene function.

7.8. Phenotypes—mutants, animal resources, RNAi and transgenics

So far the possibilities have mostly focussed on the molecules and signalling pathways active during chicken development. But the key advantage of the chicken embryo is easy access to all stages of development. When combined with the new tools and the genome sequence, we should see an increase in gene function studies in vivo. This will exploit existing and new mutants, create mutant phenotypes by transgenics or more likely by RNAi techniques.

7.9. Access to information—databases and WWW sites

Finally, the wealth of information and possibilities can be overwhelming and unless its easy to use will not be used. To ensure the full exploitation of all these new opportunities the information on the chicken genome and all the tools must be accessible. The tools themselves are being made available by the laboratories that have developed them and by central facilities (e.g. see www.ark-genomics.org). The information on the chicken genome is already out there on the genome browsers and these will serve as the basis for access to other sources of information (phenotypes, QTL, expression patterns, etc.).

7.10. Integration o f biological knowledge—the ChickNET community

Most important of all—the developmental biologists and others interested in the chicken as an experimental organism need to get organised and be willing to exchange tools and information. AvianNET (http://dev.chicken-genome.org/) is one organisation that tries to encourage this ethic (Burt and Pourquié, 2003). The aim of *AvianNET* is to serve as a gateway to a Community with an interest in the chicken genome, developmental biology, genetics, biodiversity, immunology, physiology, etc. and links to other species and tools with a shared biological interest. The long-term goals for *AvianNET* are:

- To be the community resource for the chicken genome
- To facilitate the integration of genetic, genomic, functional information in chicken
- To facilitate the use of the chick as a model for other birds and human development
- To serve the needs of the chicken research community

We would encourage all developmental biologists (not just those using the chicken but also those working on other model organisms such as mice, *Xenopus* and Zebrafish, a lot can be learned by comparative embryology) to join AvianNET (See http://www.chicken-genome.org/ and Fig. 1 for more information) and share in this new, exciting era of chicken biology.

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