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The Chick: A Great Model System Becomes Even Greater

Commentary

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Summary

The chick embryo has a long and distinguished history as a major model system in developmental biology and has also contributed major concepts to immunology, genetics, virology, cancer, and cell biology. Now, it has become even more powerful thanks to several new technologies: in vivo electroporation (allowing gain- and loss-of-function in vivo in a time- and space-controlled way), embryonic stem (ES) cells, novel methods for transgenesis, and the completion of the first draft of the sequence of its genome along with many new resources to access this information. In combination with classical techniques such as grafting and lineage tracing, the chicken is now one of the most versatile experimental systems available.

The First 2300 Years

Embryonic development is a tremendously complex process, which has fascinated man since the beginning of history. How does fertilization result in the formation of a complete, independent individual? Where is the information for this complexity encoded, and what mechanisms ensure that it is decoded appropriately? To answer these fundamental questions, science has made use of a number of "model systems," each with different advantages in that they allow various experimental approaches to different extents (Table 1). The most important metazoan model systems for studying development currently include the nematode *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster*, a few species of sea urchin (mainly *Strongylocentrotus purpuratus* and *Lytechinus variegatus*), the zebrafish *Danio rerio*, the South African clawed toad frog *Xenopus laevis*, the chicken *Gallus gallus*, and the mouse *Mus musculus*. Of these, the chicken was the first to be used for developmental investigations.

The chicken egg is such a common and accessible source of embryos that it attracted the interest of the ancient Egyptians as well as of Aristotle, who opened eggs at different stages of incubation to examine the progression of development. Until well into the 19th century, observations of chicken embryos at different stages were used to support either of the two theories of the raging debate between preformation (the adult is preformed in miniature from the time of fertilization or even earlier, and just grows) and epigenesis (the embryo increases in complexity and new organs form as it develops) (Needham, 1934; Stern, 2004; Wolpert, 2004). Along the way, the philosophers made many discoveries, as important as blood islands and the functional difference between arteries and veins, which were proposed to be connected to each other by capillary vessels (Harvey, 1628). The existence of the latter was later confirmed with the aid of a simple microscope by Malpighi, who also discovered (despite his preformationist convictions) the existence of the neural groove (neural tube) and the somites and that the beating of the heart began even before the blood started to form (Malpighi, 1672, 1675).

Subsequent progress closely followed new technical advances. Improved microscopes and early attempts at sectioning allowed the discovery of the germ layers (Pander, 1817; von Baer, 1828) and the first indications of interactions between them, which later led to the concept of induction. After the mid-1800s, the new innovation was the introduction of numerous selective dyes for staining and more sophisticated methods for sectioning, which sprouted a new generation of comparative histologists (mainly in Germany, including von Ebner, Hensen, Rauber, Koller, and Remak) who quickly generated a comprehensive description of the changes in structure of the embryo throughout development. Many of the modern concepts and the names of anatomical components of the embryo are due to the work of these pioneers, whose keen powers of observation combined with their curiosity to establish the first mechanistic insights into how development might occur (Table 2).

By the end of the 19th century, embryology was born again. Wilhelm Roux and his followers realized that carefully designed experimental manipulations that disturb development can provide information about the developmental potential of cells in the embryo, far beyond the speculations that had previously been attached to static histological observations. These studies were quickly applied to many species and led to detailed fate maps, formal definition of concepts such as regulation, induction, commitment, and competence, and the clear notion that development depends upon the flow of instructive signals between different cell populations. Around the same time (c. 1910), Thomas Hunt Morgan was building the discipline of developmental genetics and introducing the fruit fly as a system—the combination of Roux's "experimental embryology" (*Entwicklungsmechanik*) with Morgan's genetic analysis signaled the birth of modern developmental biology.

The chick joined the systems amenable to experimental embryology fairly early in the game. A few pioneers (Rawles, Fell, Rudnick, Gräper, Wetzel, Adelmann, Pannett, and others) perfected embryo and cell culture and microsurgical and fate mapping methods that made the approach accessible to chick embryos. One of the landmarks of the era is a series of stunning stereoscopic time-lapse films revealing the movements of labeled cells in living, intact embryos from the beginning of gastrulation to the laying down of organ primordia (Gräper,

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Table 1. Some Strengths and Weaknesses of the Main Developmental Model Systems

Organism	Ploidy	Full/Fine Staging System?	Experimental Embryology		Single Cell Tracing (Lineage or Fate Mapping)	Forward Genetics		Loss of Function (Gene Targeted)		Gain of Function		ES Cells	Genome Sequenced?
			Pregastrula	Postgastrula		Spontaneous Mutations	Induced Mutations	Somatic Cells	Germline	Whole Embryo	Targeted (Time and Space)		
C. elegans	2n	yes	+/–	no	yes	yes	yes	yes (RNAi)	no	no (?)	no (?)	no	yes
Drosophila	2n	+/–	no	+/–	no (+/–)	yes	yes	yes	yes	yes	yes	no	yes
Zebrafish	pseudo-tetraploid	no	yes	+/–	yes	yes	yes	yes (MO, dom.neg.)	no	yes	no	no	yes
Xenopus	pseudo-tetraploid	yes	yes	no	yes (early)	no	no	yes (MO, dom.neg.)	no	yes	+/– (Dex.)	no	+/– (*tropicalis* soon)
Chick	2n	yes	yes (from blastula)	yes	yes	yes	no	yes (MO, siRNA, dom.neg.)	no	+/–	yes	yes	yes
Mouse	2n	no	+/–	+/–	+/–	yes	yes	yes (Cre/Lox)	yes	yes	yes	yes	yes

1929), which also included observations of the behavior of isolated embryo fragments. In 1930, the highly influential figure of C.H. Waddington entered the field and over the next 10 years systematically explored the regulative ability, inducing powers, and competence of early embryos and parts thereof, the mechanisms of left-right asymmetry, and interactions between cell layers leading to control of the direction of cell movements and to the induction of the nervous system and placodes (Stern, 2000). He discovered, among many other things, mesoderm (primitive streak) induction by the extraembryonic endoderm (hypoblast) and that Hensen's node is the amniote organizer. The following decades saw Waddington's disciple Michael Abercrombie attempt to establish rules for the behavior of isolated cells, which led to the discovery of contact inhibition and other principles that laid the foundations of modern cell biology (Abercrombie, 1967, 1977), and Ruth Bellairs's finding that the definitive (gut) endoderm arises from the epiblast through the primitive streak during gastrulation (Bellairs, 1953). Transplantation experiments defined the zone of polarizing activity (ZPA) and the apical ectodermal ridge (AER) as critical signaling regions directing limb development (Saunders, 1948; Zwilling and Hansborough, 1956; Saunders and Gasseling, 1968; Tickle, 2004). It is also during this period that the quail-chick chimera technique was introduced as a powerful method to follow the migration and differentiation of cell populations in intact embryos (Le Douarin, 1973), which led to comprehensive knowledge on the origins and fate of the neural crest (which had been discovered in the chick embryo by His as early as 1868), the discovery of the hemangioblast, and many aspects of neural tube patterning (Dieterlen-Lievre and Le Douarin, 2004; Le Douarin, 2004).

Between the 1940s and mid-1970s, however, experimental embryology lost some momentum. It was followed by yet more morphological observations, applying to embryos the newly introduced techniques of electron microscopy and monoclonal antibodies to generate more anatomical descriptions at the ultrastructural and molecular levels. However, these studies led to few new insights into molecular mechanisms of development. The next major landmark was the introduction of recombinant DNA technology, which attracted a large number of molecularly trained scientists to turn their attention to the study of embryonic development. In the 1980s, the frog *Xenopus laevis* became a very attractive system for the newcomers because its large egg allows injection of constructs directly into the fertilized egg, because the early stages of development are not accompanied by any increase in embryo volume (and therefore there is no significant dilution of the injected construct), and because the phenotypic consequences can be assessed quickly and easily. However, the chick was slower to follow—most of the molecular studies were limited to analysis of gene expression in normal or manipulated embryos. A few labs, however, combined experimental embryology methods with molecular markers and other methods and continued to make very important discoveries. Among these were the finding that a subdivision of the somites along their rostrocaudal axis guides growing motor nerves and neural crest cells and generates segmentation in the peripheral nervous

Table 2. Some Major Concepts due to Work on Chick Embryos

Date	Concept	Discoverer(s)
1628	function of arteries and veins, proposed existence of capillaries	Harvey
1672–1675	neural tube, somites, capillaries	Malpighi
1817–1828	germ layers (ectoderm, mesoderm, endoderm)	Pander, von Baer
1868	the neural crest	His
1911	viruses cause cancer (Rous Sarcoma Virus)	Rous
1929	gastrulation cell movements (Polonaise)	Gräper, Wetzel
1932	extraembryonic endoderm (hypoblast) regulates embryo polarity/mesoderm induction	Waddington
1932	hemangioblast proposed (common precursor of endothelium and blood cells)	Murray
1932–1937	Hensen's node is the amniote organizer	Waddington
1936	first genetic map for the chicken	Hutt
1948–1968	Apical Ectodermal Ridge controls limb outgrowth	Saunders
1953	gut endoderm is derived from the epiblast via the primitive streak	Bellairs
1956	Zone of Polarizing Activity patterns the A/P axis of the limb	Zwilling, Saunders
1960–1968	T- and B-lymphocytes	Miller, Good, Glick, Claman
1964–1970	provirus hypothesis and reverse transcriptase	Temin
1967	contact inhibition	Abercrombie
1970	importance of extraembryonic endoderm (hypoblast) in head development	Eyal-Giladi and Wolk
1975 onwards	hemangioblast demonstrated	Dieterlen-Lièvre, Le Douarin
1976	first cellular oncogene (*c-src*)	Bishop and Varmus
1984	somites control segmentation of peripheral nervous system	Keynes and Stern
1985–1987	retinoic acid as a limb morphogen	Tickle, Eichele
1988	the notochord patterns the dorsoventral axis of the spinal cord	Van Straaten
1989	rhombomeres are embryologically and functionally important	Lumsden and Keynes
1991	DT40 cells undergo frequent homologous recombination	Buerstedde
1993	Sonic hedgehog patterns the spinal cord (D/V) and specifies motor neurons	Jessell
1993	Sonic hedgehog is the ZPA morphogen	Tabin
1995	a genetic cascade patterns the dorsoventral axis of the limb	Tabin
1995	a genetic cascade regulating left-right asymmetry	Tabin, Kuehn, Stern
1997	oscillating gene expression during somitogenesis	Pourquié

system (Keynes and Stern, 1984; Kuan et al., 2004), proof of a segmented organization of the hindbrain into rhombomeres (Lumsden and Keynes, 1989; Fraser et al., 1990; Lumsden, 2004), the discovery that the notochord induces the floor plate and ventral identity (including motor neurons) in the developing spinal cord (van Straaten et al., 1985; Jessell, 2000; Price and Briscoe, 2004), and the finding that oscillating cycles of gene expression precede somite formation (Palmeirim et al., 1997; Pourquie, 2004).

At this point, a technical limitation precluded misexpression of genes into chick embryos: the cells are too small for direct, routine injection of constructs. This had limited misexpression studies to secreted factors, which could be applied either via soaked inert beads (Summerbell, 1983) or through grafts of transfected heterologous cells into chick embryos. The first new technique to overcome this was retroviral vectors. In combination with other techniques, it led to identification of key molecules emanating from the ZPA and the AER and those that initiate limb outgrowth and dorsoventral patterning (Morgan et al., 1992; Riddle et al., 1993; Laufer et al., 1994; Tickle, 2004). This was followed by the discovery of a first set of four genes whose expression is left-right asymmetric during normal development and demonstration of their critical roles in establishing left-right asymmetry (Levin et al., 1995; Raya and Izpisua Belmonte, 2004). Replication-deficient retroviral vectors have also been used very effectively as cell lineage tracers (Gray et al., 1988) as well as for delivery of small interfering RNAs (siRNA) to silence gene expression (Devroe and Silver, 2002).

This was the state of affairs near the turn of this century—the chick was an old, venerable system that had contributed many key concepts and embryological facts that turned out to be generally applicable, but it was becoming unfashionable because, with only a few exceptions, it was difficult to perform more sophisticated gain- and loss-of-function experiments that could be combined with experimental embryology, which remained its main strength. Furthermore, sequencing of its genome was a low priority while efforts were directed to yeast, nematode, fly, *Fugu*, mouse, and human. All this changed over the last few years, and especially in 2004.

But we shouldn't leave behind these 2300 years of history without at least a brief mention of the chick as a genetic system, as well as some of its contributions to fields other than developmental biology. Domestication of the jungle fowl over several thousands of years of civilization led to the establishment of numerous strains (inbred to various degrees) that were selected to be particularly good meat producers or productive egg layers. In the process, several mutant lines were identified and some of them preserved (unfortunately, however, many of them are now in danger of being lost). Some of these mutations affect important developmental processes, for example, the *talpid* mutation produces interesting defects in limb development (Goetinck, 1964; Ede and Agerbak, 1968), but although there have been many studies characterizing the phenotype, the molecular nature of the gene has not yet been elucidated. A list of mutations (including stocks considered "at risk") can be found at http://www.grcp.ucdavis.edu/publications/indexa.htm (see Table 3). Indeed, the chicken was the first agriculturally important species for which a genetic linkage map was constructed, as long ago as 1936 (Hutt, 1936).

Table 3. Some Useful Chick Resources on the Web

Web Site	Purpose	Notes
http://www.chicken-genome.org	AvianNet—chicken information network	a portal to other databases, genomic tools, discussion groups, etc.—maintained by Dave Burt at Roslin Institute
http://www.thearkdb.org/chicken	gene mapping data, integrated databases	maintained by Roslin Institute—US mirror site: http://iowa.thearkdb.org/
http://www.chicken-genome.org/resources/databases.html	list of most databases	list and links to most current databases—compiled by Roslin Institute
http://www.ensembl.org/Gallus_gallus	chick genome browser (EBI/Sanger)	includes access to multispecies comparisons, gene families, gene prediction tools, etc.
http://genome.wustl.edu/projects/chicken/	Wash U. genome project	summary of chicken genome sequencing project
http://www.genome.ucsc.edu/cgi-bin/hgGateway	Univ. California Santa Cruz chick genome browser	alternative genome browser for chicken and other genomes, includes different features from Ensembl
http://www.ncbi.nlm.nih.gov/genome/guide/chicken	chick genome browser	NCBI access to chick genome, with good cross-database links
http://www.chick.umist.ac.uk	chick EST database	340,000 ESTs from 64 libraries, includes BLAST facilities, GO-searching, SNP variants, RNAi design tool, and other features
http://chicken.genomics.org.cn/index.jsp	chick SNP database	lists variations of sequences in different chicken strains—maintained by Beijing Genomics Institute
http://genetics.hpi.uni-hamburg.de/dt40.html	DT40 EST database and resources	Jean-Marie Buerstedde's project on bursal genes and DT40 cells, including SAGE data
http://www.chickest.udel.edu	Univ. Delaware EST project	40,000 ESTs from UD cDNA library collection
http://www.tigr.org/tigr-scripts/tgi/T_index.cgi?species=g_gallus	TIGR GgGI database	integrated information on ESTs, genes, loci, expression, function, etc.
http://hbz7.tamu.edu/homelinks/phymap/chicken/chick_home.htm	physical map, genetic map, and BAC library tools and resources	developed by Hongbin Zhang (Texas A& M U) and Jerry Dodgson (Michigan State)
http://www.zod.wau.nl/vf	BAC libraries and BAC-related resources, chick AceDB, and ChickFPC	Wageningen Univ. (Netherlands)
http://bacpac.chori.org/	BAC and PAC libraries	
http://poultry.mph.msu.edu/resources/Resources.htm	chromosome linkage map, microsatellite markers/primers, BAC libraries, etc.	includes information and primer-pair kits for microsatellites covering the chick linkage map for QTL mapping and other applications
http://www.vjf.cnrs.fr/image/chicken/	chicken IMAGE—disease and immunity-related gene information	CNRS, France
http://geisha.biosci.arizona.edu	gene expression database for ESTs	Parker Antin's database of EST expression data at early stages. Good for quick reference of likely expression sites
http://genex.hgu.mrc.ac.uk	(under development) chick anatomy atlas + expression database	the link currently points to the mouse atlas site, but this will be integrated with chick anatomical and gene expression data for known genes. Will be good for high-confidence, carefully curated, and 3-dimensional data on expression and cross-species comparisons
http://udgenome.ags.udel.edu/~cogburn/	functional genomics project	Cogburn lab, Univ. Delaware
http://animalscience.ucdavis.edu/AvianResources/ and http://www.grcp.ucdavis.edu/publications/indexa.htm	avian genetic resources	list of current chicken genetic stocks and resources "at risk"—Mary Delany at Univ. California Davis
http://www.chicken-genome.org/resources/affymetrix-faq1.htm and http://www.affymetrix.com	EST microarrays (under development)	Affymetrix to release whole-genome microarrays soon based on sequences in GenBank and dbEST

Some of the most momentous contributions, however, have been in the fields of virology, cancer, and immunology. These include the discovery of Rous Sarcoma Virus (RSV), which first established a causal link between viruses and cancer (Rous, 1911) (Nobel Prize 1966). This culminated in the isolation of the first cellular oncogene (*c-src*) from chicken cells by Varmus and Bishop (Nobel Prize 1989) (Stehelin et al., 1976) and the discovery of reverse transcriptase and the formulation of the "DNA provirus hypothesis" by Temin, which elucidated the mechanism by which RNA viruses become incorporated into their host cells (Nobel Prize 1975, with Baltimore and Dulbecco) (Temin, 1964; Temin and Mizutani, 1970). In immunology, the discovery of T- (thymus) and B-lymphocytes (the latter named after the Bursa of Fabricius, a bird-specific organ, but this type of lymphocyte of course also exists in mammals) made by several laboratories in the 1960s (mainly Miller, Good, Glick, and Claman; see Miller, 2004) remains of huge importance today.

A New Beginning: A Great System Becomes Even Greater

In the last few years, the classical approaches have been enormously enriched by three major technical advances: the introduction of new methods for gain- and loss-of-function and promoter analysis, the isolation of embryonic stem (ES) cells and development of new methods for transgenesis, and the sequencing of the chicken genome and establishment of numerous new electronic resources.

New Methods for Gain- and Loss-of-Function and for Promoter Analysis

In 1997, Muramatsu et al. (1997) explored the possibility of misexpressing genes in a temporally and spatially controlled way in chick embryos using a variety of nonviral methods and discovered that in ovo electroporation is a very efficient technique. Subsequent studies, mainly in Nakamura's laboratory, refined this technique, making it possible to introduce expression constructs very efficiently into regions of any size, at almost any position, and at any stage of development (Nakamura et al., 2004). Expression vectors were meanwhile being optimized in several laboratories, and there is currently a good selection of these for different applications. For misexpression regardless of cell type, a most effective construct (pCAβ-IRES-GFP) contains the β-actin promoter and CMV enhancer, a polylinker for inserting the desired gene followed by an internal ribosome entry site (IRES) and green fluorescent protein (GFP) (Yaneza et al., 2002; Sheng et al., 2003). This construct allows misexpression to be physically targeted to a group of cells of any size or shape at any time in development, or even to a single cell, by controlling the shape and position of the electrodes (it should be noted, however, that mesenchyme and other loose tissues are more difficult to target than epithelia because most of the current tends to pass between cells in the former). It is also possible to direct expression to specific subsets of cells within the electroporated region by replacing the β-actin promoter with a cell type-specific one, or one containing an inducible element. The approach allows very rapid and efficient gain-of-function studies with full control of the position and timing of expression of the gene of interest. It also allows loss-of-function studies not only by introducing inhibitory or dominant-negative constructs, but also can be used to transfect either fluorescein-labeled morpholino oligonucleotides (MO) (Sheng et al., 2003; Nakamura et al., 2004) or siRNA-producing DNA constructs (Bron et al., 2004; Nakamura et al., 2004), either alone or together with a rescuing construct into selected cell populations (Sheng et al., 2003). The main advantage of this method over the injection of MO or mRNA in *Xenopus* is the spatial precision with which the MO or construct can be introduced and that it can be made to direct expression or knockdown at any stage of development.

In addition to gain- and loss-of-function experiments, electroporation can also be used to analyze the activity of promoters in vivo (Yu et al., 2000; Uchikawa et al., 2003, 2004). With GFP as a reporter, results can be obtained within a few hours by direct observation of the living embryos and changing patterns of gene expression followed by time-lapse filming. Here, the chick presents significant advantages over the more established methods of promoter analysis in mouse, which requires the production of transgenic animals. Using this technique, a very compelling analysis of the regulatory regions driving chicken *Sox2* expression was recently carried out, which uncovered 25 distinct enhancer elements with different stage- and tissue-specific activities, most of which are conserved in mouse and human (Uchikawa et al., 2003). Without a doubt, this technology has transformed the chick embryo into a very powerful system indeed. Current research in several labs is now perfecting other methods such as sonoporation, improved lipofection, and biolistics (the "gene gun"), which may hold further promise for the future.

Stem Cells and Transgenesis

Embryonic stem (ES) cells have proved extremely useful for developmental studies in the mouse, where they have been used not only as a tool for the generation of transgenic animals by homologous recombination and for the construction of chimeras, but also to study various aspects of cell differentiation and the roles of various genes in cell commitment. However, true ES cells with the potential to contribute to all somatic tissues as well as to the germline have to date only been generated from mouse blastocysts—for some reason it has proved very difficult to generate ES cell lines from other species and to demonstrate their totipotency beyond doubt. Although this has not yet been achieved fully in the chicken, recent advances presage that it may become possible very soon. Dissociation of embryonic cells at the "blastula" stage (stage X), soon after egg laying, followed by culture under special conditions that favor cell proliferation and inhibit differentiation, can generate cell lines that continue to proliferate for many passages in vitro. When these cells are introduced into host blastoderms at a similar stage (which can be done easily by blunt injection with a syringe), they are found to contribute to all somatic cell types tested, and at least for early passages they can also contribute to the germline, albeit with low frequency that can be improved somewhat by γ-irradiation of the recipient embryo (Carsience et al., 1993; Petitte et al., 2004). While in culture, the ES cells can be manipulated genetically, as they are amenable

to transfection using various methods. Homologous recombination is very frequent in the leucosis virus-induced B-cell line DT40 (Buerstedde and Takeda, 1991) but has not yet been efficiently achieved in chicken ES cells, which has so far precluded the production of germline transgenic birds using this method.

Other methods have met with more success (Sang, 2004). Injection of DNA directly into fertilized eggs generates germline transgenics at very low frequency, and attempts have also been made to produce stably transfected primordial germ cells that can be introduced into the circulation, from where they will colonize the gonads. More recently, however, a lentiviral vector was shown to be an efficient transducer when injected into eggs and to be capable of generating germline transgenic animals expressing GFP at high frequency (McGrew et al., 2004). Although this method does not permit reverse genetics approaches to the extent that homologous recombination does in mice, it is more efficient and simpler than methods currently used to produce transgenic zebrafish or *Xenopus tropicalis* embryos.

Progress in this area has been so rapid that it is now only probably a matter of time before these methods can be combined or perfected sufficiently to achieve efficient germline transmission of transgenes that may well include homologous recombination. However, I believe that for developmental studies, ES cells that can be manipulated genetically and can contribute to all somatic tissues will be particularly useful for somatic cell genetics experiments (rather than whole-animal genetics, as in mouse), particularly when combined with the obvious advantages of the chick for transplantation, filming, and labeling studies, offering the opportunity to perform experiments as elegant as those currently being carried out in *Drosophila*.

The Chicken Genome

The first major advance toward sequencing the chicken genome was made in March, 2003, through the production and sequencing of 64 cDNA libraries from 21 different embryonic and adult tissues by a consortium led from the Roslin Institute in Edinburgh, UMIST in Manchester, and the universities of Dundee and Nottingham. This led to 339,314 EST sequences that clustered into 64,760 gene bins that were assembled into 85,486 contigs, representing about 10,000 genes (Boardman et al., 2002). To this were added sequences from other EST libraries from other projects, which generated close to 500,000 EST sequences.

In March, 2004, the first draft sequence of the complete chicken genome was released, complemented by the production of BAC libraries and a BAC-based physical map (Wallis et al., 2004), identification of numerous single nucleotide polymorphisms (SNPs) (International Chicken Polymorphism Map Consortium, 2004), and the compilation of a genetic map for the chicken genome. Genome sequencing was accomplished by a consortium led from Washington University (St. Louis, MO), who used a shotgun approach to obtain 6.6× coverage of the genome of a single female Red Jungle Fowl bird (considered to be the common ancestor of all extant domestic fowl). A first annotated version followed in May, and an initial analysis is now being published in *Nature* (International Chicken Genome Sequencing Consortium, 2004) along with the two other papers mentioned above. The chicken genome has a haploid content of 1.2×10^9 base pairs divided among 40 chromosomes (including the sex chromosomes W and Z, the female being heterogametic—WZ). Cytogenetic studies coupled with the recent genome maps allow identification of 31 of these, the remaining 9 being among minichromosomes that, despite their small size, contain about twice the gene density found in the 9 macrochromosomes (Burt, 2004). The draft sequence comprises 1.05×10^9 bases (91% of the genome), of which 933 Mb (89%) can be mapped onto identifiable chromosomes. The chicken genome is very compact indeed (Figure 1), compressed by 40% with respect to the human and mouse genomes, yet it is currently predicted to contain between 20,000 and 23,000 protein-coding genes (at the lower end of the range displayed by mammalian genomes) as well as 571 noncoding RNAs (including many fewer pseudogenes than mammals) from more than 20 gene families. There is a particularly low frequency of retrotransposon-derived sequences, and, unlike any other vertebrate genome studied so far, there is a complete absence of SINEs (small, nonautonomous retroposons derived from structural RNAs). The coding genes show very high similarity to human genes, and only two chicken protein families (Pfam) are absent from the human genome, while a further 21 are absent from *Fugu*. Some of the innovations of the chicken are obvious (including, for example, genes involved in the biology of the eggshell, feathers, etc.), and some of the genes that are not represented are similarly obvious (including milk protein genes, salivary associated proteins, hair keratins, and enamel proteins). There are also some surprises. For example, it was thought that birds have a poor sense of smell, yet the genome sequence predicts the existence of 283 distinct olfactory receptors (a similar number to that found in humans). On the other hand, taste receptors are greatly expanded in mammals (International Chicken Genome Sequencing Consortium, 2004). Perhaps the peasants of Piedmont and the Périgord should now explore the potential of chickens as truffle hunters?

This is the first nonmammalian amniote genome to be sequenced, and since release of the first annotated version in May, 2004, it has already started to prove a very valuable resource, particularly for comparative genomic analysis, especially the identification of conserved noncoding regions, which is greatly aided by the evolutionary position of the avian lineage with respect to other vertebrates as well as by the compactness of the chicken genome. Bird and mammalian lineages are thought to have diverged about 310 Myr ago, so this genome fills a much-needed gap between the mammalian genomes sequenced so far (human, chimpanzee, mouse, and rat, with cow and dog to follow soon) and other vertebrate genomes (*Fugu* and *Tetraodon*, with zebrafish and *Xenopus tropicalis* to follow soon). Unlike teleost fishes and many anuran amphibians, however, the genome of the chicken has not undergone a recent duplication, and in most cases there is 1:1 correspondence between homologous genes in mammals and birds, which includes a high level of sequence conservation in intronic and flanking noncoding regions likely to contain important regulatory elements. The sequencing consortium estimates that at least 70 Mb of the newly

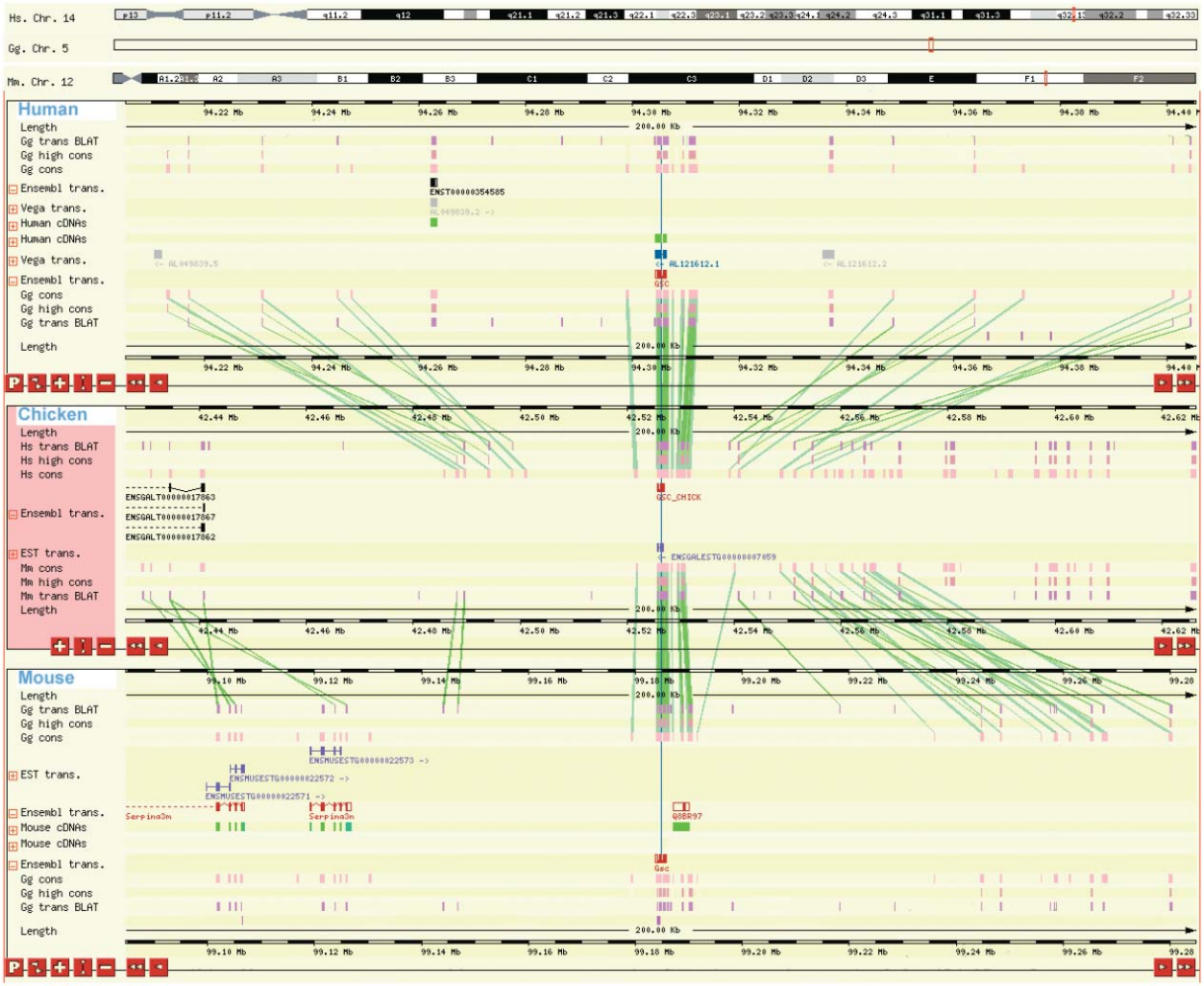

Figure 1. Alignment of the Region of Chicken Chromosome 5 Containing the *goosecoid* Gene with Its Syntenic Regions in the Human and Mouse Genomes

The syntenic regions are found on human chromosome 14 and mouse chromosome 12. Numerous conserved noncoding sequence blocks are shown in pink, linked by green lines across the three species. Note the compactness of the chick genome as compared to its two mammalian counterparts. Also, as is commonly found with these comparisons, the organization and conservation between human and chick blocks is greater than between chicken and mouse. Here there appears to have been a local transposition in the mouse genome (left in the figure). Obtained using MultiContigView from http://www.ensembl.org/Gallus_gallus/ and searching for goosecoid.

obtained chicken sequence is likely to encode functional, conserved elements, and there are long blocks of conserved synteny between chicken and mammals and a very low rate of chromosomal translocations (International Chicken Genome Sequencing Consortium, 2004).

For developmental biologists, as well as for evolutionary biologists, immunologists, and many others, these features of the chicken genome are great news. One of the main persuasive reasons for undertaking the sequencing of the chicken genome was the expectation that its compact genome and unique evolutionary position with respect to mammals would greatly facilitate the identification of putative regulatory regions, and this is already amply demonstrated even at the current level of resolution of the assembly, which still has some 10% of coding sequences missing (some due to incomplete sequences or ambiguous assemblies or unassembled BACs, others for unknown reasons). The draft sequence also makes it possible for the first time to design tools to study alternative splicing (including gene conversion for immunologists working on DT40 cells), to design siRNAs and morpholinos for loss-of-function studies, and many other powerful applications. Of course, production of a first assembly is quickly followed by a shift of priorities in sequencing centers and funding agencies. For it to be truly valuable, both for those working on the chicken and for those whose interests are primarily in human biology and medicine, it is imperative that the project is not abandoned here and that some further funding is made available to finish the sequencing and assembly and to provide fuller annotations.

The Future

The new technologies and resources now usher a new era for the chick as a system for developmental, genetic, immunological, evolutionary, molecular, physiological,

and many other studies. It will now be particularly efficient to identify candidate regulatory elements by comparing noncoding regions between chicken and mammals, which can then be tested very quickly by electroporation into intact embryos. Loss-of-function and gain-of-function constructs can be designed and quickly introduced by the same method. Even the current methods for producing transgenic birds (Sang, 2004), although still primitive in comparison with the mouse, should already allow the production of transgenic lines of birds expressing a reporter in particular tissues, which will be of great value for numerous applications, as well as the generation of ES cells whose differentiation can be studied in culture. But for the developmental biologist, the largest strides will probably continue to be made by combining the old and new technologies: cell lineage analysis and transplantation together with genetic manipulations.

Completion of the draft genome sequence was quickly accompanied by an unprecedented (for the chick) amount of intergroup communication. The chick was until now the only main model species that did not host a regular system-based meeting, and the laboratories of chick developmental biologists were much more self-contained than those working on most other species. Just before release of the EST sequences, the first Chicken Genome meeting was held in Manchester, and since then others have followed in the Sanger Centre and Stowers Institute in Kansas City to discuss the new technologies, and the first Chicken Developmental Biology meeting is currently being planned for the Spring of 2006 (probably in Spain, organized by Marianne Bronner-Fraser, David Burt, Olivier Pourquie, Kate Storey, Cheryll Tickle, and myself). Without a doubt, what used to be a field comprising many isolated workers who rarely talked is now well on the way to becoming an interactive community. This can even be seen by the web sites of individual laboratories, which increasingly feature links to resources and to each other to stimulate cross-fertilization and sharing of technologies and resources. The chicken has now come of age as a major model system for biology, medicine, and agriculture. Let us hope that funding agencies (and the referees who provide them with input) will make available the required funding to allow the valuable genetic resources to be preserved, as well as for the sequencing project to be taken to completion.

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