

Preface

The chick embryo – past, present and future as a model system in developmental biology

The embryo of the domestic fowl (*Gallus gallus*) holds the record as the animal with the longest continuous history as an experimental model for studies in developmental biology, spanning more than 2 Millenia. Throughout this time, it attracted great naturalists, artists, philosophers, and pioneers of biology and stimulated them to think about the most fundamental questions on generation and life like no other organism has ever done, except the human.

The ancient Egyptians are documented as having opened hens' eggs at different periods during incubation to observe the progress of embryo development, and by around 300 BC Aristotle undertook careful studies of the morphology of the embryo (as much as he could without the aid of magnifying devices); this can be considered as the first 'scientific' study of embryo development and his work referred to by his followers right up to the 19th Century (Needham, 1959). After the mediaeval 'Dark Ages', the resurgence of an interest in Anatomy and embryo development in the Renaissance attracted figures including Leonardo Da Vinci (1452–1519), Ulisse Aldrovandi (1522–1605) and Hieronymus Fabricius ab Aquapendente (1537–1619) to return to the study of the embryo within the egg. At this time the debate between Preformation and Epigenesis was starting to gather momentum and the chick embryo was often a reference to which subscribers to either camp resorted to support their favourite theory. As an attempt to contribute to the debate, William Harvey (1578–1657) observed chick embryos at early stages of development and concluded that the heart was the first functioning organ to develop in the embryo. By observing the motion of the blood through the heart and early vessels, he discovered the circulation of the blood and understood the function of arteries and veins.

The ensuing 200 years or so were accompanied by an increase in the number and the detail of anatomical studies of the embryo, each time enriched by a new technical advance. The introduction of histological sectioning and of selective staining methods allowed Pander and von Baer to start to understand the significance of germ layers in development. These pioneers also started to ask questions

about causality in development—what mechanisms are responsible for such stereotyped development? Further and increasingly careful histological studies followed throughout the 19th Century, with the most important contributions being made by Rauber and Hensen and culminating in a beautiful histological atlas by Mathias Duval (1889). By the end of the 19th Century, experimental embryology (*Entwicklungsmechanik*) started to replace simple histological observation as it became clear that principles could only emerge from experimental manipulation of the embryo, but the initial advances were mainly made through work in other organisms (sea urchins and amphibians for 'embryo-embryonic regulation' and induction, marine invertebrates for lineage studies, *Drosophila* for developmental genetics) and the chick was a little slower in catching up. But there were some salient chick studies at this time, including Graeper's spectacular 3-d stereo time-lapse movies of embryos labelled with spots of vital dyes to follow cell movements (made in 1926, published in 1929 and unrivalled to the present day), which revealed the cell movements preceding and during gastrulation and Waddington's cross-species transplants of primitive streak and node and his hypoblast rotations which led to the first evidence that extraembryonic endoderm (hypoblast) plays a role in positioning the embryonic axis (Waddington, 1932, 1933), as well as consolidating the concept that Hensen's node is a source of signals for neural induction in both mammals and birds. Likewise it is not widely known that Waddington pioneered an experimental approach to understanding the development of left–right asymmetry (Waddington, 1937; Waddington and Cohen, 1936).

In the last 50 years, the chick embryo has contributed some of the most important general concepts in vertebrate developmental biology. This includes the discovery of the mechanisms that pattern the vertebrate limb and the ZPA and AER as signalling regions therein (John Saunders, Lewis Wolpert, Cheryll Tickle), the demonstration of the movements and fates of the neural crest by Le Douarin, the discovery that the notochord (and Sonic hedgehog signalling from it) regulates dorsoventral polarity and the location

of different neuronal subpopulations within the neural tube by van Straaten and Jessell, the importance of somites in controlling segmentation of the peripheral nervous system (Keynes and Stern) while the central nervous system is autonomously segmented (Lumsden), the discovery of T- and B-cells and the hemangioblast by Le Douarin and colleagues, and many more. As molecular biology merged into developmental biology, it was in the chick that the first 'dynamic' gene expression pattern (Hairy-1 and Lunatic Fringe) was discovered to be correlated with somite formation (Pourquie) and that the first four genes regulating left–right asymmetry were found (Sonic hedgehog, Nodal, Activin-receptor IIA and HNF3 β ; Levin et al., 1995). And the DT40 cell line has also turned out to be a superb system to study genetic recombination and the origin of immunological diversity. The first section of this volume honours some of these momentous contributions, with a series of reviews written by some of those who made them.

It seems extraordinary that despite such a history, in more recent days there have been signs of 'anti-chick' racism by institutions hiring new young faculty, reviewers of grant applications and even some reviewers of submitted manuscripts. It is not unusual to see advertisements by institutions seeking experts on *C. elegans*, *Drosophila*, zebrafish and occasionally mouse, but I have never seen a search specifically for a chick embryologist. One reason is obviously our current interest in genes and their functions, but it also shows a disregard for the value of the search for 'fundamental biological principles' as an important endeavour. Vertebrates have some 30,000 genes but only relatively few really important principles governing cell behaviour. I would be very surprised if it turned out that all of the latter have already been defined clearly enough, and it is these contributions that will have more lasting influence on our knowledge than the discovery of the roles or mode of action of a few more genes. Furthermore gene function cannot be studied without a solid background in the cellular principles of development, and it is not until these principles are fully revealed that some of the gene functions will be recognised or become experimentally tractable.

In the last half-decade or so, however, the chick has come of age. Its enormous power as a system for experimental embryology (transplantation, lineage studies and following cell movements in vivo) has now been complemented by exciting new technical advances allowing sophisticated genetic manipulation as well. First, the discovery that even very large and complex constructs can be introduced by electroporation into specific cell populations at precisely controlled times in development by the group of Nakamura has revolutionised the experiments that can now be done, since the approach can be used both for time/space-controlled gain-of-function and for loss-of-function (using dominant-negative constructs, siRNA and morpholino oligonucleotides). This approach can also be used rapidly to map even large and extremely complex regulatory regions, as demonstrated for the *Sox2* promoter by the group of Hisato

Kondoh (Uchikawa et al., 2003). Second, a draft sequence has now been generated covering the entire chicken genome by Washington University (St Louis), accompanied by confirmation that this animal possesses about the same number of genes as humans but is extremely compact and with an amazing level of conserved synteny with mammals. The evolutionary position of the chick (as an amniote) makes this a unique resource that will soon greatly speed up the characterisation of gene regulatory regions as well as rationalisation of gene homologies. Third, methods have now been introduced for deriving chick Embryonic Stem (ES) cells from early embryos and manipulating them genetically (by Petite, Etches and Pain). These cells can contribute to all somatic lineages and recent advances in this technology make it likely that a method will soon be found to allow them to contribute to the germ line as well. Finally, new viral vectors have been designed which allow almost routine generation of stable, transgenic lines of birds (see cover picture and review by Helen Sang in this volume). Together, these new technologies, honoured in the second part of this volume, promise to give the chick embryo a huge new impetus as a leading system for developmental biology and many other areas.

In particular, organisms traditionally considered as 'genetic' like *Drosophila*, *C. elegans*, zebrafish and the mouse lend themselves to identification of germ line mutations which affect one or more specific developmental processes, because all cells in the embryo contain the mutation from the beginning of development. However, it is more difficult to unravel the role of a gene in a particular process if the gene has multiple functions at different times in different places. 'Conditional mutagenesis' techniques such as Cre–Lox recombination in the mouse and the Gal4–UAS system in the fly allow tissue specific and time-restricted analysis, but the process can be complicated, dependent on having promoters that will drive expression in the correct tissue and/or at the desired time, and especially difficult for analysing the roles of two or more genes. Coupling the new techniques with cell manipulation in the chick offers exciting opportunities for very targeted studies of gene function in specific developmental processes, unrivalled in other species. And the possibility of genetic manipulation of chick ES cells, even without them contributing to the germ line, will soon allow somatic cell genetics to be performed in vivo with at least the same level of sophistication as in the zebrafish. The next few decades will no doubt demonstrate that this old and very distinguished friend is by no means old-fashioned or no longer useful.

References and further reading

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