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eChickAtlas: An Introduction to the Database

Frances Wong¹, Monique C. M. Welten², Claire Anderson³, Andrew A. Bain¹, Jiahui Liu³, Michael N. Wicks⁴, Gordana Pavlovska², Megan G. Davey⁵, Paula Murphy⁶, Duncan Davidson⁴, Cheryll A. Tickle², Claudio D. Stern³, Richard A. Baldock⁴, and Dave W. Burt^{1*}.

¹Division of Genetics and Genomics, The Roslin Institute and Royal (Dick) School of Veterinary Studies, The Roslin Institute Building, University of Edinburgh, Easter Bush Campus, Midlothian, United Kingdom.

²Department of Biology and Biochemistry, University of Bath, Claverton Down Road, Bath, United Kingdom.

³Department of Cell and Developmental Biology, University College London, Gower Street, Anatomy Building, London, United Kingdom.

⁴MRC Human Genetics Unit MRC IGMM, University of Edinburgh, Western General Hospital, Crewe Road, Edinburgh, United Kingdom.

⁵Division of Developmental Biology, The Roslin Institute and Royal (Dick) School of Veterinary Studies, The Roslin Institute Building, University of Edinburgh, Easter Bush Campus, Midlothian, United Kingdom.

⁶School of Natural Sciences, Zoology Building, Trinity College Dublin, Dublin, Ireland.

*Corresponding author

Professor David W. Burt

Division of Genetics and Genomics, The Roslin Institute and Royal (Dick) School of Veterinary Studies, The Roslin Institute Building, University of Edinburgh, Easter Bush Campus, Midlothian, United Kingdom.

Phone: +44 (0)131 651 9216

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Fax: +44 (0)131 651 9105

Email: Dave.Burt@roslin.ed.ac.uk

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Abstract

The precise control of gene expression is critical in embryonic development. Quantitative assays, such as microarrays and RNA sequencing, provide gene expression levels for a large number of genes, but do not contain spatial information. In contrast, *in situ* methods, such as *in situ* hybridisation and immunohistochemistry, provide spatial resolution, but poor quantification and can only reveal the expression of one, or very few genes at a time. Furthermore, the usual methods of documenting the results, by photographing whole mounts or sections, makes it very difficult to assess the three-dimensional (3D) relationships between expressing and non-expressing cells. Optical projection tomography (OPT) can capture the full 3D expression pattern in a whole embryo at a reasonable level of resolution and at moderately high throughput. A large database containing spatio-temporal patterns of expression for the mouse (e-Mouse Atlas Project, EMAP, www.emouseatlas.org) has been created, incorporating 3D information. Like the mouse, the chick is an important model in developmental biology and translational studies. To facilitate comparisons between these important model organisms, we have created a 3D anatomical atlas, accompanied by an anatomical ontology of the chick embryo and a database of gene expression patterns during chick development. This database is publicly available (www.echickatlas.org).

Introduction

The *eChickAtlas* resource is modelled on *eMouseAtlas* (Baldock et al, 2003; Christiansen et al, 2006; Armit et al, 2012) and consists of three main components: 1) a collection of reference model embryos at a wide range of stages of development from laying (stage EKG-I) (Eyal-Giladi and Kochav, 1976) to stage HH26 (Hamburger and Hamilton, 1951) in two (2D) and three dimensions (3D), 2) an abstract anatomical ontology for the chick embryo containing the major structures, regions and tissues, 3) a growing collection of data on gene expression

patterns in 2D and 3D for chick embryos at the above-mentioned range of stages. Other databases of gene expression in the chick embryo already exist, such as the Gallus Expression in Situ Hybridization Analysis (*GEISHA*) database (Antin et al, 2007; Darnell et al, 2007), which offers a repository of gene expression patterns at many stages of chick development.

GEISHA aims to cover as many genes as possible, but image annotations are basic with limited anatomical references and it relies on data from a mixture of sources (published, submitted and generated by the *GEISHA* team). In contrast, *eChickAtlas* aims to provide more detailed images assembled into a curated database with consistency in staging and image formats, accompanied by annotations. For many stages the *eChickAtlas* includes 3D images of both standard anatomy and gene expression patterns, with the capability to overlap the expression patterns of different genes, create virtual sections along any plane and to link with other species such as the companion Atlas for the mouse. We envisage the chick and mouse atlases to enrich each other and to offer a very important resource for comparative studies.

At early stages of development (until neural tube formation, about stage HH9-HH10), the chick embryo is relatively flat, so these embryos have been imaged in 2D. As the embryo develops, body folding and other morphogenetic events increase the thickness of the embryo, which makes topological relationships between different structures more important, but also more difficult to evaluate. Optical projection tomography (OPT) is therefore used to image them in 3D to achieve a more complete data set.

The atlas of reference model embryos covers stages EGK-X – HH9 in 2D and HH10 – HH26 (inclusive) in both 2D and 3D (figure 1). All embryos have been selected for the best match of the specified stage. They have gone through the same *in situ* hybridisation protocols as used

for the gene expression database but without RNA probe to ensure that these reference embryos, and the corresponding anatomical annotations, are directly comparable to those used for mapping gene expression, compensating for any shrinkage or other artefacts generated by the *in situ* hybridisation procedures. This reference library includes a fully defined staging system, following the standard tables of (Eyal-Giladi and Kochav, 1976) for pre-primitive streak stages (in Roman numbers, I-XIV) and (Hamburger and Hamilton, 1951) for later stages, as well as an anatomical ontology. The current ontology (figure 2) is used to annotate the atlas of gene expression and incorporates all terms of the EMAP mouse anatomy (adjusted for mouse-chick differences and mainly corresponding to development up to HH26 in the chick) to allow cross-reference to the chick, as well as from the standard atlas of avian anatomy (Bellairs and Osmond, 1998). The anatomy database enables queries of the site of gene expression. More specifically, it allows integrated queries to be made across the gene expression data that has been annotated at different levels of resolution. For example, one set of expression data annotated as in the limb and another annotated as in the apical ectodermal ridge (AER), a region of the limb, will both be retrieved by a query for genes which are expressed in the limb. It is envisaged that future developments will include extension of the user interface to allow more complex and sophisticated queries, both within the chick and between chick and mouse, and potentially other species.

The *in situ* hybridisation protocols are standardised for consistency across the database. A web-based submission interface validates data consistency, tested within the project and is ready for external users. It includes management of a probe database, which is a useful search tool allowing a specific probe or assay to be found and to explore gene expression patterns across embryonic stages. More specifically, a probe is defined as the RNA *in situ* hybridisation probe used and an assay is equivalent to a single description of a single gene expression pattern

in a single specimen (with any accompanying images and experimental details). To date, about 200 probes and 1000 assays have been submitted to the database, which contain both 2D (stages EGK-X – HH9) and 3D data (stages HH10 – HH26) (figure 3). The current database has been created from submissions by the laboratories of the authors of the present paper, but it is envisaged that in the near future, submission will be opened to other laboratories, provided that a standardised set of guidelines is followed, and the data subjected to careful curation by the Chick Atlas consortium.

The purpose of the submission interface is for data submission. If users simply want to browse the database for gene expression data, this can be easily done via the gene expression atlas portal. This portal allows queries for a specific gene/protein, developmental stage, assay ID or assay type. It will also allow more than one search term per query. For example, users can query a specific gene/protein at a particular developmental stage by adding an extra search field within the same query. This is very useful if users know exactly what information they want to view. Other users may want a basic overview of the gene expression data. In this case, one can browse all gene expression data by entering an asterisk “*” in the gene/protein query box and results will show all current gene expression data within the database. This results list can be sorted into alphabetical order according to gene name by clicking on the “Gene/Protein” header at the top of the column. Gene expression data is continually submitted into the database for users to view and the gene expression atlas portal will be developed and expanded in the future for users to carry out other types of queries.

The initial *eChickAtlas* database has been populated with patterns of expression of a number of genes isolated from a series of microarrays designed to enrich for genes that are differentially expressed in signalling regions at different stages of embryogenesis. Other genes were selected

based on their representation in the mouse database or their significance in recent studies of the developing chick (Fisher et al, 2008; Bangs et al, 2010; Fisher et al, 2011; Welten et al, 2011).

This initial data set will enable development and expansion of new tools to allow comparative studies, which will hopefully start to reveal common and divergent regulatory mechanisms.

Furthermore, this analysis will allow exploration of developmental heterochrony (a developmental change in the timing of events, leading to changes in size and shape), which will be critically important for understanding differences between mouse and chick. This may in turn enrich our understanding of how the avian and mammalian lines emerged during evolution and about the molecular mechanisms responsible.

OPT can capture the full 3D expression pattern in a whole embryo at a resolution of about 12 microns (voxel sampling size; where a voxel is a volumetric element representing a value on a regular grid in 3D space) at a moderately high throughput. This resolution does not detect single cells, but is sufficient to reveal small, multicellular regions of expression. As an example, figure 4 shows the expression pattern by 3D rendering and virtual sectioning of one of the genes isolated from the above mentioned series of microarrays, PKIG (*protein kinase (cAMP-dependent, catalytic) inhibitor gamma*). Previous studies have shown that virtual sectioning by OPT is comparable to histological sections (Sharpe et al, 2002). The OPT approach is less time-consuming compared to the labour intensive, low-throughput method of full 3D reconstruction of histological sections assayed for *in situ* hybridisation. In summary, 3D data can be produced by OPT without histological sectioning and can be visualised in 3D, graphically manipulated and, in future with the development of current tools, spatially mapped onto the reference models. Software tools already in use in the Mouse Atlas resource, allowing different genes to be spatially mapped onto the same embryo, can be

adapted and optimised for 3D mapping of chick data. Spatial mapping will aid investigations into co-expression patterns of different genes, allowing their pathways to be elucidated.

This resource is publicly available (www.echickatlas.org) with a direct link to *eMouseAtlas* (www.emouseatlas.org) where cross comparisons to the mouse can be made. Direct links allowing cross comparisons with other species can be developed in the future. These links will be very valuable for comparative developmental biology. It is also anticipated that gene expression data from other avian systems, such as duck, quail and turkey, may be added to the *eChickAtlas* database. The creation, development and maintenance of this database come at a time when online resources are the first point of reference for information, not only with scientists and students but with the general public. All *eChickAtlas* activities will be in close collaboration with national and international groups and consortia, making this a true community resource.

METHODS

Embryos

Fertilized White Leghorn and Brown Bovan Gold chicken eggs were obtained from Henry Stewart (Lincolnshire) and incubated in a humidified incubator at 37°C for the appropriate length of time for the desired developmental stage according to (Eyal-Giladi and Kochav, 1976) or Hamburger Hamilton (Hamburger and Hamilton, 1951), as appropriate. Embryos were excised, transferred to ice-cold Phosphate Buffered Saline (PBS) (0.02 M Phosphate, 0.15 M NaCl), cleaned of extra-embryonic membranes and fixed in 4% (w/v) ice-cold paraformaldehyde (PFA) overnight. Embryos were then put through a graded methanol series at 4°C; ending in 100% methanol and stored at -20°C until use.

Probe synthesis

CHRD (*chordin*) was linearised with EcoRI (NEB) and transcribed with Sp6 RNA polymerase (Roche) to produce an antisense probe. PKIG (ChEST 318i8) was acquired from ARK Genomics. This EST clone was in the pBluescript II KS+ vector, which was linearised with NotI (NEB) and transcribed with T3 RNA polymerase (Roche) to produce an antisense probe.

Whole mount RNA in situ hybridisation

Whole mount RNA in situ hybridisation for early (EGK-X – HH14) stage embryos was performed as described in (Stern, 1998; Streit and Stern, 2001). Whole mount RNA in situ hybridisation for mid (HH15 – HH20) and late (HH21 – HH26) stage embryos was carried out using a protocol adapted from (Wilkinson and Nieto, 1993). Details of all protocols used can be found at www.echickatlas.org/submission/protocols. Images were captured using a Zeiss Lumar V12 SteREO microscope with an AxioCam high resolution camera attached and using the AxioVision imaging software system (release 4.8).

Optical Projection Tomography

Embryos were prepared for optical projection tomography as described in (Fisher et al, 2008) and scanned following the protocol set out in (Sharpe et al, 2002). A Bioptonics 3001 OPT scanner (www.bioptonics.com) or a prototype OPT scanner was used to scan all embryos. Images were processed for 3D rendering and visualised using Amira 5.4.1 software.

Acknowledgments

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Figure 1. Hamburger Hamilton (Hamburger 1951) stage selection: Stages HH21-HH26, are shown in the time-line. A: HH22 has been selected and highlighted (green), and three-dimensional (3D) reconstruction data (pink) and a Hamburger and Hamilton stage description (blue) are also available for this embryo. Both these functions are linked to resources available online. B: virtual section of the 3D reconstruction data from the HH22 selected embryo from A. Tool bar on the left allows users to move through the embryo, change the magnification and view different virtual sections of the embryo in different planes.

Figure 2. Chick specific anatomical terms forming part of an ontology tree created using OBO Edit. The wing has been highlighted. All terms connected to the wing have been expanded to demonstrate the anatomical components which make up the wing.

Figure 3. Example of an assay submission into the database, extracted from the submission interface. An *in situ* hybridised embryo is displayed, along with its three-dimensional (3D) reconstructed data. The 3D reconstructed data are displayed as still images and movies in different views (whole embryo and virtual sections). Information on the gene expression pattern and gene annotation is also displayed. This example shows the gene expression pattern of CHR1 (chordin) at HH18.

Figure 4. Virtual three-dimensional (3D) gene expression patterns of PKIG (*protein kinase (cAMP-dependent, catalytic) inhibitor gamma*) at HH21 captured by optical projection tomography (OPT). PKIG expression was captured using bright field OPT, where the level of staining throughout the embryo is shown to range from blue (low) to green (high). A: views of volume rendering of the 3D OPT data; B: virtual section showing expression in the neural tube and strong expression in the somites.

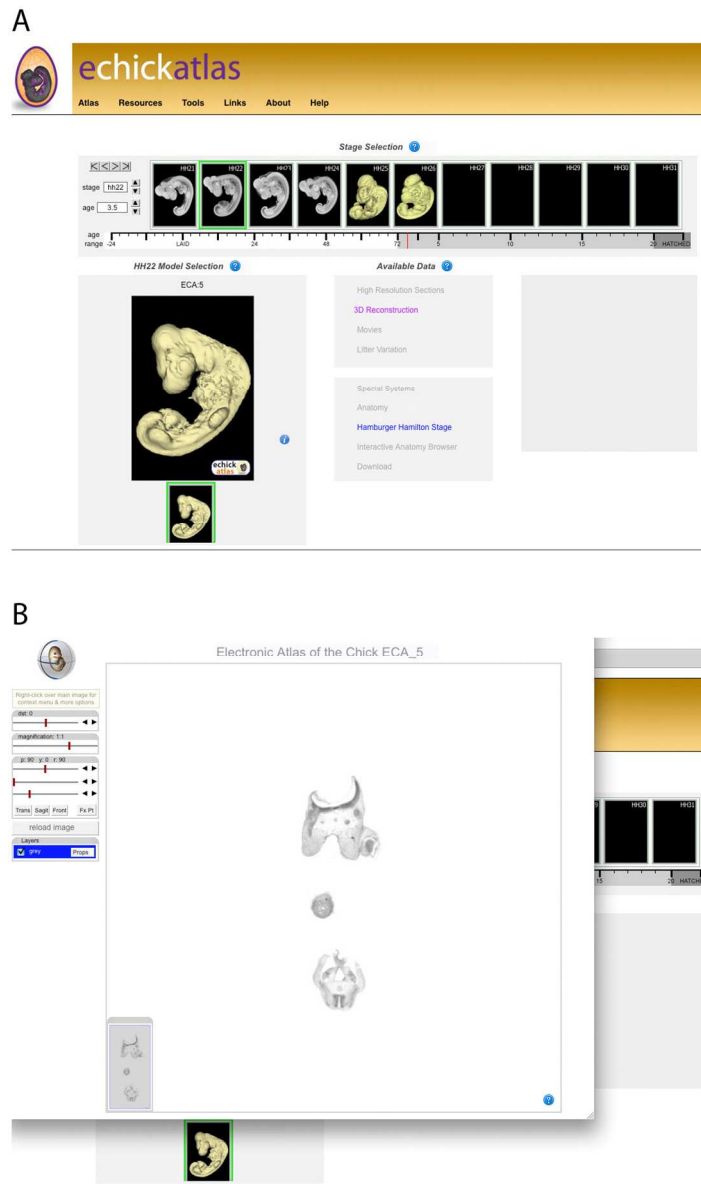


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391x650mm (72 x 72 DPI)

A

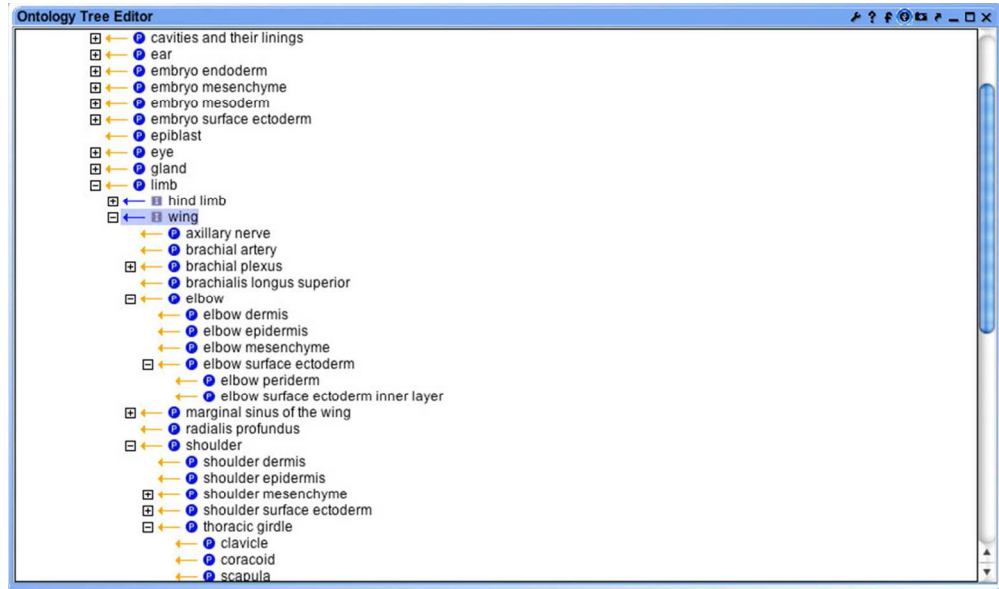


Figure 2. Chick specific anatomical terms forming part of an ontology tree created using OBO Edit. The wing has been highlighted. All terms connected to the wing have been expanded to demonstrate the anatomical components which make up the wing.
302x177mm (72 x 72 DPI)

Accepte

IMAGE FILES

Local Zip File or Comment:

Number of Image Sets: 1

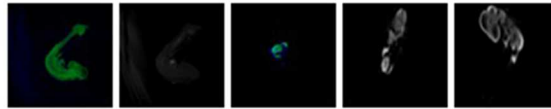
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Image Quality: High

DATA



Images:



Movies:



EXPRESSION PATTERN DESCRIPTION

Detected In: distal tip of notochord

Not Detected In:

Possibly Detected In:

ANNOTATIONS

Annotation	ID:	TMP:1304
	Name:	notochord
	Strength:	Strong
	Pattern:	Graded
	Note:	distal tip

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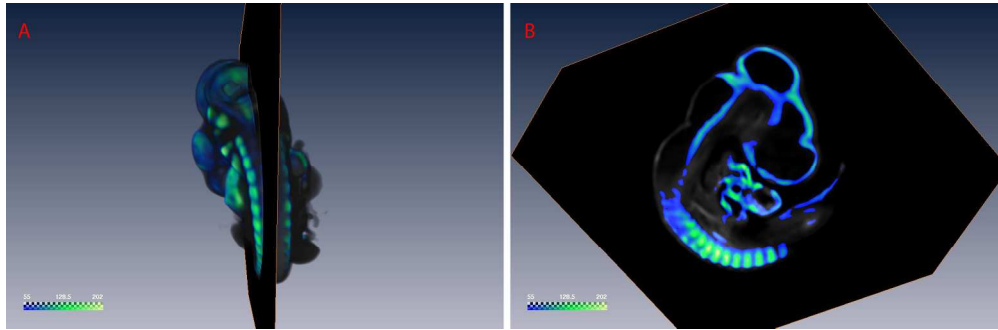


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800x260mm (72 x 72 DPI)

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