Meeting report

Chickens get their place in the sun

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A report on the International Chick Meeting 'The Chick as a Model Organism: Genes, Development and Function', Barcelona, Spain, 11-14 April 2007.

The chicken was the first model organism in animal biology, with a history of investigation stretching over 2,300 years and an unmatched contribution to biological concepts, especially in developmental biology and immunology. In the late 20th century, it lost some of its status to more genetically manipulatable organisms, but recent advances in avian transgenesis and the availability of its complete genome sequence have put the chicken back in the mainstream. The first international chick meeting held in Barcelona recently brought together researchers from around the world who use the chicken as a model system, and was designed to give as many young researchers as possible the opportunity to communicate their science. The conference covered a wide variety of subjects, including immunology, development, genetics, genomics, bioinformatics and nutrition. Here I summarize a few of the advances in developmental biology reported at the meeting.

The importance of timing

Somite formation is a key developmental process that depends on timing. Along the antero-posterior axis of the embryo, discrete blocks of mesoderm, the somites, arise in periodic fashion from an unsegmented tissue, the pre-somitic mesoderm (PSM), and subsequently differentiate into vertebrae and the skeletal muscles of the trunk. The periodicity of somite formation is controlled by a molecular oscillator, also known as the segmentation clock, which is represented by the cyclic expression of a large number of genes. Olivier Pourquie (Stowers Institute for Medical Research, Kansas City, USA) described microarray experiments in chick, mouse and zebrafish to find those genes whose cyclic expression in the PSM is conserved. Surprisingly, the only conservation found was in components of the Notch signaling pathway. This raises the possibility that the core oscillator in somite formation is associated with Notch signaling, but it is also possible that the Notch signaling-related oscillating genes are only the readout of a still-elusive molecular oscillator that operates upstream of Notch.

The duration of a signal can also be crucial in determining cell fate. The lens of the eye and the olfactory epithelium of the nose arise from embryonic ectodermal structures known as placodes. Lena Gunhaga (Umea University, Sweden) has investigated the role of bone morphogenetic protein (BMP) signals in the generation and specification of these placodes using chick embryonic tissue explants. She found that progenitors of both the olfactory and lens placodes are specified during gastrulation (the phase in the early embryo when the three primary germ layers and basic body plan are established) and that BMP signaling is necessary and sufficient to induce both olfactory and lens placodal character. But how is lens versus olfactory character specified? Different levels of BMP signaling do not affect the ratio of olfactory and lens placodal cells produced, but prolonged exposure to BMP signaling leads to lens specification. This result shows that the duration of exposure of progenitor cells to patterning signals can play a pivotal role in the specification of their fate.

The introduction of genes into chick embryos by *in ovo* electroporation is a powerful tool for assigning gene function. However, expression of the introduced genes usually lasts for only a few days, as the transgenes are not stably integrated into the genome. Yoshiko Takahashi (Nara Institute of Science and Technology, Nara, Japan) presented a new method for stable integration and inducible expression of electroporated transgenes, which utilizes the *Tol2* transposon system from a fish (the medaka). The gene to be introduced is flanked by sequences recognized by the Tol2 transposase, and the gene construct is electroporated together with a transposase-encoding plasmid. Takahashi described

the stable integration of a green fluorescent protein (GFP) transgene in several tissues, including somitic mesoderm, retina and limb bud. GFP expression also persisted to late stages of development. To achieve conditional expression, the *Tol2* system was combined with the tetracycline-inducible (tet-on) system. In this case, the introduced gene remains inactive until the administration of doxycycline, an analog of tetracycline. Three plasmids are co-electroporated. The first carries a constitutively expressed Tol2 transposase gene. The second contains the transgene under the control of a tetracycline-responsive element (TRE), with the whole construct flanked by *Tol2* sequences to ensure integration. The third plasmid encodes a constitutively expressed gene for the rtTA transcription factor (which is required for expression from the TRE promoter), again flanked by *Tol2* sequences. This system allows the expression of introduced genes to be induced late in embryogenesis, and thus enables the study of genes implicated in later stages of organogenesis.

Chick limb development

The chick has long been a model for studying all aspects of limb development. Ana Certal (Instituto Gulbenkian de Ciéncia, Oeiras, Portugal) discussed the role of the potassium channel Erg1 in the regulation of cell proliferation and apoptosis during limb development. Erg1 transcripts are present early during limb initiation in both mouse and chick. Following limb induction, Erg1 is expressed in the progress zone of the limb bud, and later in the interdigital spaces and the phalanges during digit development. Knockdown of Erg1 expression by RNA interference (RNAi) at early stages of limb development leads to either severely truncated or smaller limbs compared with untreated limbs. When Erg1 is knocked-down later, in the interdigital areas of 5-day-old embryos, the interdigital tissue did not regress, leading to "webbed" digits (syndactyly). Erg1 is thus a key player in limb induction, outgrowth and digit patterning. Certal's group is now investigating the functional relationships between Erg1 and proteins known to be important in these processes.

The protein Sonic hedgehog (Shh) is expressed in the zone of polarizing activity (ZPA) in the posterior ectoderm of the developing limb bud. Shh forms a posterior-to-anterior gradient and acts as a morphogen for the patterning of the limb bud. Marian Ros (Universidad de Cantabria, Santander, Spain) focused her talk on the role of BMP signaling in controlling the expression domain of Shh in the posterior and distal limb mesenchyme. Blocking of BMP signaling results in the expansion of the Shh expression domain in more proximal regions of the mesenchyme. Conversely, beads soaked in BMP implanted into the mesenchyme block Shh expression. Although the blocking effect is very fast (1-2 hours), protein synthesis is likely to be required. Further experiments showed that Wnt5a is a strong candidate for acting downstream of BMPs in the positioning of the Shh expression domain.

Neural crest cell development

The vertebrate neural crest is comprised of cells of ectodermal origin that are generated between the prospective neural tissue (the neural plate) and the adjacent epidermis and migrate to numerous sites in the body following neurulation (neural tube formation) to give rise to a great diversity of cell types. Marianne Bronner-Fraser (California Institute of Technology, Pasadena, USA) presented recent results that describe an interconnected genetic network that controls the different stages of neural crest formation. Bronner-Fraser's group had previously shown that the signal protein Wnt6 is necessary and sufficient for induction of neural crest cells from neural plate, and that BMP signaling is also involved. More recently, she has found evidence that neural-crest specification involving Wnt and BMP signals takes place at an earlier stage, during gastrulation. She has found that Pax7, a paired homeodomain-containing transcription factor essential for neural crest specification, is expressed in chick epiblast during gastrulation and defines the region of the epiblast that is specified as neural crest. Wnt and BMP signals are required for Pax7 expression prior to neurulation. At later stages, Pax7 activates characteristic neural-crest genes such as *Bmi1*, a member of the polycomb-repressive complex, which is important in keeping the cells in an undifferentiated state, and *Sox10*, a gene essential for neural crest-cell migration. Promoter analysis of *Sox10* showed that it is a direct target of Pax7. Current work in Bronner-Fraser's group is focused on the recognition of downstream target genes of *Snail2* which is esstential in neural crest development.

Tissues derived from neural-crest cells include skeletal and connective tissues in the head. Nicole Le Douarin (Collège de France, Paris, France) stressed that head structures are formed from neural-crest cells that do not express any Hox genes. Removal of the Hox-negative domain produced embryos that did not develop a head. Misexpression of Hox genes in this area produced the same result, indicating that the absence of Hox gene expression is a key factor in the pathway responsible for neural-crest-derived head structures. Le Douarin described transplantation and gain- and loss-of-function experiments showing that expression of the fibroblast growth factor FGF8 in the branchial arches is necessary and sufficient for head-structure formation. This expression is inhibited by BMP signaling, and she speculated that the absence of Hox gene expression in neural crest cells either prevents the expression of BMP family members or leads to the expression of the BMP antagonist Gremlin. Either way, BMP signaling is repressed, which allows the expression of FGF8 and the formation of the head structures.

The neurons of the enteric nervous system (ENS) originate from neural-crest cells migrating along the gut from the vagal and sacral regions of the embryo. Vagal neural-crest cells can migrate along the entire gut and contribute most of the ENS. Sacral neural-crest cells give rise to a small number

of cells in the post-umbilical gut. Amanda Barlow (Institute of Child Health, University College London, UK) described a careful comparison between sacral and vagal neural-crest cells for the expression of genes known to be required for ENS formation, with the aim of determining the basis for this difference. There were no qualitative differences in either mRNAs or proteins, but Ret, a receptor tyrosine kinase essential for cell migration and the development of the ENS, was found to be expressed at significantly higher levels in the vagal neural-crest cells. When Ret was overexpressed in sacral neural-crest cells, these cells also colonized the gut in large numbers and earlier in development.

The release of the first annotated version of the chick genome in May 2004 has proved a tremendous resource for more integrative biology using the chick. Analysis of gene expression in the chick embryo using electroporation-introduced transgenes now takes less time than the generation of transgenic mice. The proposed BirdBase, an avian database compatible with other model organism databases, will develop and enhance avian biology resources, support research and education, and integrate genomic and biological information across different platforms. Overall, the meeting showed that what was once a research field populated by isolated labs is now a developing and interactive community.

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