MANIPULATION OF THE AVIAN GENOME

Organizers: Robert Etches and Ann Gibbins

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Manipulation of the Avian Genome

A Cellular Approach to the Avian Genome

CI 001 THE ANATOMY OF REPRODUCTION IN BIRDS WITH EMPHASIS ON POULTRY, Murray R. Bakst, Avian Physiology Laboratory, USDA/ARS, Beltsville, MD 20705

Approaches for insertion of genetic material into the avian genome vary and utilize both the male and female gamete and the fertilized ovum. To fully appreciate these technological advances, a foundation in the basic anatomy and physiology of avian reproduction is required. To begin, the strategy of reproduction in birds differs considerably from other domesticated animals. If fertile, the ovoviviparous bird oviposits an egg containing an embryo in the gastrulation stage of development. Fertilization had taken place about 26 hours before oviposition with participating sperm that had been inseminated days or weeks before fertilization. These sperm were subjected to intense selection by the oviduct prior to storage within discrete tubules located in the distal portion of the oviduct. It is the capacity to slowly but continuously release sperm from the oviducal sperm-storage sites which assures an adequate number of sperm at the site of fertilization in the absence of repeated inseminations.

Anatomically, the testes are situated ventral to the cranial lobes of the kidneys. The excurrent ducts, consisting of rudimentary epididymides and tightly coiled ductus deferens, serve as conduits for semen between the testes and the cloaca. There are no accessory sex glands. However, semen is diluted with a lymph-like fluid derived from the tumescent phallus at ejaculation. Morphologically, sperm are narrow and elongated possessing a head and tail region which lack the regional specializations and outer dense fibers, respectively, observed in livestock sperm.

In the female, only the left ovary and oviduct develop. With a hen in egg production, the largest in the hierarchy of megalecithal oocytes ovulates within 45 minutes of the previous oviposition and is grasped by the fimbriated region of the oviduct. Here, if sperm are present, the ovum may be fertilized. Regardless if fertilized or not, the ovum progresses through the five segments of the oviduct where it accrues albumen, a shell membrane, and shell over the next 26 hours.

Artificial insemination (AI) is an integral part of the breeder sector of the turkey industry and is a common practice in research programs addressing problems in poultry reproduction. Basically, a two-step procedure (semen collection and insemination) most AI programs now include diluting the semen immediately after collection with a cell culture-like medium referred to as an extender or diluent. By using the appropriate semen diluent under the appropriate conditions semen can be stored for several hours without affecting fertility.

CI 002 A GENETIC APPROACH TO PHYSIOLOGY

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The genome of domestic birds has been extensively manipulated during the past two thousand years to produce approximately 230 recognized breeds of chickens and countless commercial strains each with a set of physiological attributes that makes them unique. These stocks have been developed because they possess a morphological phenotype that is aesthetically appealing or useful. In the future, selection for birds with the appropriate growth rate, feed conversion, egg production and other morphologically obvious phenotypes will continue to improve the productivity of the commercially important strains and breeds of poultry. In the future, however, the range of traits that can be considered for manipulation will be expanded as the genetic control of physiological processes is elucidated. Manipulation of the avian genome, therefore, depends upon the ability to recognize and understand the physiological processes of interest at the DNA level and to access the avian genome to facilitate changes to the coding sequences for these processes. The Colloquium is devoted to the study of these two phenomena beginning with the formation of the haploid female gamete, its exposure at ovulation and the restoration of diploidy at fertilization. The structure of the genetic material is essential information in developing a strategy to manipulate the avian genome and the technologies that have been used and developed to introduce changes into the genome will be considered. Finally, the physiological consequences of the genetic changes that might be introduced need consideration and discussion. It is the objective of the colloquium to provide a forum for a comprehensive discussion of each of these issues that impact on our ability to manipulate the avian genome.

CI 003 FROM CLEAVAGE TO AXIS DETERMINATION IN BIRDS, Hefzibah Eyal-Giladi, Department of Zoology, Hebrew University, Jerusalem 91904, Israel.

During the early development of the chick, a series of consecutive morphogenetic events is responsible for the imprinting of bilateral symmetry on the initially radially-symmetric germ. The symmetrization is mainly expressed by the gradual establishment of the posterior section of the marginal zone (MZ) as the dominant source of a population of hypoblastic cells, which are responsible for the induction of the primitive streak (PS) in the epiblast. In a series of experiments, the morphogenetic movements as well as the inductive potential of the MZ belt, have been studied. Posterior MZ cells have been demonstrated to move anteriorly into the forming hypoblast, while lateral MZ cells show a very limited movement. The MZ belt functions as a coordinative system, in which there is a very subtle equilibrium of inductive and inhibitory affects, which ensures the induction of a single PS. The posterior MZ cells seem to gain their inductivity only after leaving the MZ belt, and crossing Koller's sickle into the hypoblast.

A Chromosomal Approach to the Avian Genome

CI 004 THE GENETIC MAP AND AVAILABILITY OF DIVERSE GENETIC MATERIAL, J. James Bitgood, Department of Poultry Science, University of Wisconsin-Madison, Madison, WI 53706.

Interest in the chicken gene map is reviving, partly due to the availability of new molecular and biochemical procedures for mapping. This is occurring when genetic resources available for mapping may be declining.

Some portions of the chicken gene map are growing rapidly, while there is no activity in other parts. The Z chromosome and chromosome 1 remain the most thoroughly mapped. Recent additions to the maps of these two chromosomes have been made using techniques other than classical test crosses. Cytogenetic procedures, biochemical studies to include alloantigen tests and protein polymorphisms, and molecular techniques such as in situ hybridization have all contributed.

These varied techniques need to be applied in collaborative studies to maximize mapping information. Then when a gene is located in a chromosome region, it can also be ordered in a linear relationship with other genes. Tests are underway that integrate several disciplines. Reference populations being created from diverse genetic backgrounds will be useful for identification of syntenic groups. Additional studies will be needed to integrate these groups into the current map.

Many biomedical researchers have identified chicken genes and use them in their studies. These investigators generally have little interest in the animal itself, or in gene mapping. These laboratories may not participate in a gene mapping program, but the information that they generate will be valuable.

Rapid advances may be possible in the near future, but diversified stocks may not be as available. As university poultry geneticists retire there is evidence that they will not be replaced by poultry specialists, let alone personnel trained in avian genetics. Stocks characterized over a number of generations have recently been lost, and more will be lost. The recent reduction in the number of poultry science departments will aggravate the situation. There is no repository for potentially useful genetic material such as exists for plants. It has been suggested that hobby flocks are not the reservoir of germ plasm that was previously thought, due to the large influence of a small number of fanciers and hatcheries specializing in breed types. Somes' Registry should be continued, and probably needs updating now; however, funding constraints cast a question on its continuance.

CI 005 CONSTANT AND VARIABLE FEATURES OF AVIAN CHROMOSOMES, Stephen E. Bloom and Mary E. Delany, Department of Poultry and Avian Sciences, Cornell University, Ithaca, NY 14853. -- Chromosome complements in avian species include both constant and variable features that must be considered in studies to map genes, identify sites of integration of foreign DNA, and to track donor cells to tissue sites in chimeras. Detailed cytogenetic studies in the domestic chicken are restricted to primarily the first 10 chromosome pairs, each of which is morphologically distinct. High resolution banding permits analyses of yet smaller chromosomes (e.g., No. 1 - 15). The primary constitutive features of the avian genome have been revealed by G-banding for structural landmarks, C-banding for constitutive heterochromatin, RBG-banding for DNA replication patterns, and in situ hybridization for localizing highly repeated sequences to centromeric and telomeric regions. Variable features of the chromosome complement and of gene expression in selected repeated gene families provide convenient metaphase and interphase phenotypes for studies in vitro and in vivo. A significant amount of genomic variability has been observed among early chick embryos (1 to 15% aberration frequency among genetic lines) including haploidy, triploidy, trisomy, and mosaicism. The inadvertent selection of an aberrant embryo as a source of cells for chimeric production could yield surprising or confusing results if cells are not karyotyped. Variability at the ribosomal RNA gene cluster generates polymorphic nucleolar (PNU) patterns in interphase cells. Polymorphic cells have one macro and one micronucleolus per cell (Pp). This phenotype is easily diagnosed in cytological preparations. We have developed chicken genetic strains with defined PNU patterns. A particular nucleolar variant is expressed in the embryo and at all other stages including adult. We have detected the PNU phenotype in stage X embryos, in embryonic tissues representing ectoderm, mesoderm, and endoderm, and in feather pulp cells from chickens. Heterozygous PNU cells should be easy to track to tissue sites when transferred to non-polymorphic recipient embryos. While development of homozygous PNU embryos (pp) is arrested shortly after stage X of development, heterozygotes (Pp) develop normally. Studies with rDNA variants should be useful for investigating the relative contributions of maternal ribosomes versus embryo-derived rRNA in early developmental processes. (Supported by grants from the NIEHS ES03499 and USDA NY157433).

Entering the Genome Through Embryonic Tissues

CI 006 THE USE OF AVIAN CHIMERAS IN DEVELOPMENTAL BIOLOGY,
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The avian embryo is accessible to sophisticated surgery during the morphogenetic and organogenetic periods. Territories of the blastodisc germ layers, organ rudiments or groups of cells can be exchanged between two embryos according either to orthotopic or heterotopic patterns. The prominent heterochromatin mass associated with the nucleolus in the quail species, used as a marker in associations of chick and quail cells (Le Douarin, 1969), has promoted the avian model to the rank of a paradigm. This stable, easily evidenced, marker has been widely used to analyze the development of nervous, immune and hemopoietic systems, and of the limb. Mapping of cell origins, fates and migration pathways, demonstrating the influence of microenvironments at the sites of arrest, were some of the achievments. Other markers have been employed in order to refine the resolution at the level of cell populations or to confirm conclusions acquired in the heterospecific system, for instance chicken MHC haplotypes, monoclonal antibodies recognizing cell lineages in only one of the two species, such as endothelial cells, or T cell subpopulations. More recently this approach has been pursued in post hatching animals to study behavior, genetic traits such as epilepsy, the function of the immune system and the mechanisms of tolerance in animals experimentally endowed with a chimeric immune system from the very first days of embryonic life. In a number of cases, the developmental rules established from avian chimeras were later confirmed using mammalian experimental models. The refinement of the possible experimental patterns should open unique perspectives for the use of avian cells transfected with foreign genes.

CI 007 ACCESSING THE GENOME USING GERMLINE CHIMERAS IN THE CHICKEN.
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During the early embryonic development of the chick, access to and genetic modification of primordial germ cells (PGCs) or their precursors would facilitate the insertion of novel genes into the avian genome. PGCs were first transferred from the germinal crescent of a donor embryo to a sterile recipient in order to demonstrate the extragonadal origin of avian germ cells (Reynaud, 1969; *J. Embryol. exp. Morph.*). Subsequently, attempts have been made to transfer PGCs at various stages of development into normal recipients to form germline chimeras (Wentworth *et al.*, 1989; *Poultry Science*; Simkiss *et al.*, 1989; *Protoplasma*). Although incorporation into the embryonic gonad has been demonstrated, recovery of the transferred genotype in planned test matings has not been reported for the chicken.

As an alternative to PGC transfer, blastodermal cells from the stage X (Eyal-Giladi and Kochav, 1976; *Developmental Biology*) embryo have been used to develop somatic and germline chimeras (Petitte *et al.*, 1990; *Development*). Recent work on the development of chimeras by blastodermal cell transfer has shown that the process of chimera formation is dependent upon the region of the stage X embryo used as a source of donor cells and that cells from earlier stages (V-VIII) can form chimeras when injected into stage X recipient embryos. In addition, blastodermal cells from embryos of various stages have been transfected with several *lacZ* reporter constructs using cationic liposomes. After transfection, stage X blastodermal cells are injected into recipient embryos, and exogenous ß-galactosidase activity can be observed in both embryonic and extraembryonic tissues. Therefore, the transfection and transfer of blastodermal cells and the subsequent development of transgenic/chimeric chickens should offer a novel means of manipulating the avian genome.

CI 008 TRANSFECTION OF CHICK EMBRYOS MAINTAINED UNDER IN VITRO CONDITIONS, Helen Sang, Clare Gribbin, David Morrice and Margaret Perry, AFRC Institute of Animal Physiology and Genetics Research, Edinburgh Research Station, Roslin, Midlothian EH25 9PS, Scotland, U.K.

An in vitro method has been developed for culture of the chick fertilised ovum through to hatch (1). This method enables access to the very early stages of development which normally occur within the oviduct. Plasmid DNA has been injected into fertilised ova prior to the first cleavage division. The fate of two reporter gene constructs has been followed by culturing embryos for up to seven days. Analysis of the results of injecting pRSVcat suggested that the injected DNA was replicated during the first 24h of development and subsequently lost (2). The results of injecting the construct pHFBGCM, which contains the lacZ gene under the control of the CMV immediate early promoter, have been followed by staining for β-galactosidase activity. Expression of the reporter gene was first seen after about 12h of development. After 24h of development (at the onset of blastulation) more than 90% of embryos contain stained cells within the area pellucida. After the primitive streak stage the number of cultures with stained cells within the embryo rather than in the extra-embryonic blastoderm, decreased substantially. After 7 days in culture only 7% of surviving embryos had stained cells within embryonic tissues. These results suggest that, if the plasmid DNA is being integrated into the host chromosomes, integration is a relatively rare event. The possibility of using the injection system as method of transient assay of genes expressed in early development is being explored.

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Manipulation of the Avian Genome

Viral and Sperm-Mediated Transfection

CI 009 SPERM CELLS AS VECTORS FOR THE GENERATION OF TRANSGENIC CHICKENS

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Rooster sperm cells were used as vectors for the introduction of foreign DNA sequences into the chicken genome. The sperm cells were diluted in a buffered solution that prolongs their viability and the diluted cells were incubated with the bacterial lacZ or chloramphenicol acetyl transferase (CAT) genes, driven by general or tissue specific promoters. Following artificial insemination of hens with the treated sperm cells, the fertilized eggs were incubated and the resulting embryos or chickens were analyzed for the presence and expression of the transgenes. Southern and polymerase chain reaction assays revealed that between 30-60% of the transfected animals contained the foreign DNA. CAT or β-galactosidase enzymatic assays were consistent with the DNA analyses but revealed that the foreign genes were expressed in a mosaic pattern. The foreign genes were transmitted through the germ line to the F1 generation.

CI 010 AVIAN RETROVIRUSES AND GENE TRANSFER INTO THE AVIAN GENOME, D. Salter, W. Payne, L. Provencher, L. Crittenden, Departs. of Micro. and Anim. Sci., MSU and Reg. Poul. Res. Lab., E. Lansing, Mi 48823; M. Federspiel, C. Petropoulos, J. Bradac, S. Hughes, NCI- Frederick Can. Res. Facility, Frederick, MD 21701. We have previous described the generation of transgenic chickens by avian leukosis virus (ALV) infection of the early blastoderm (1). Two of 23 proviral inserts were defective in the formation of infectious retrovirus: alv6 coded for subgroup A envelope glycoprotein only whereas alv11 coded for the group-specific antigen and subgroup A envelope glycoprotein (2). These defective proviral inserts are receptor interference pathogen-derived resistance (PDR) genes since cell cultures were resistant to subgroup A Rous sarcoma virus (RSV) but susceptible to subgroup B RSV. Extensive in vitro and in vivo studies of this model PDR gene will be summarized (3,4).

Replication-competent ALV retroviral vectors have been constructed for transferring and expressing foreign genes from a functional promoter in vivo independently from gag, pol and env genes (5). Embryos were infected in ovo at day of set with an ALV vector containing the chloramphenicol acetyl transferase (CAT) gene linked to chicken skeletal muscle α-actin gene promoter. Results will be presented to show that these vectors will be very useful for testing promoter-gene combinations for fidelity of expression and for in vivo targeted gene expression in poultry.

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Genetic Control of Cellular Processes

CI 011 SYNTHESIS AND DEPOSITION OF EGG-PROTEINS, Roger G. Deeley, Pamela Hoodless, Aimee Ryan and Timothy Schrader, Cancer Biology Laboratories, Queen's University, Kingston, Ontario K7L3N6, Canada

The synthesis of both yolk and egg-white proteins is regulated primarily by estrogen. Although the production of both sets of proteins is tightly coordinated, each group is synthesized in different tissues and in distinct cell types. Yolk proteins are produced exclusively in the parenchymal cells of the liver. They are secreted and ultimately deposited in the developing oocyte via interaction with a family of specific receptors. In contrast, the proteins of the egg-white are produced in tubular gland cells and secreted directly into the lumen of the oviduct. Despite the fact that several of the major yolk-protein genes are normally dormant in males they can be activated in embryos of either sex at particular stages of development by administration of estrogen *in ovo*. Thus these two groups of proteins and their cognate genes provide attractive models with which to investigate the combination of mechanisms involved in determining the tissue specificity with which they are expressed, their degree of hormonal dependence and the protein/receptor interactions that define their specific sites of deposition. Recent studies on the molecular biology of these processes will be discussed with particular reference to:1) the involvement of DNA/protein interactions in determining the ability to activate specific egg-protein genes, to define the tissue specificity with which they are expressed and the efficiency with which they are transcribed; 2) features of the promoter regions of some of the major yolk-protein genes that confer an exceptional degree of hormonal dependence; 3) the possibility of manipulating yolk protein promoters *in vivo*, and 4) their use in the production of heterologous proteins that may be targeted for deposition in the egg.

CI 012 THE MOLECULAR BIOLOGY AND GENETIC CONTROL OF GROWTH IN POULTRY, C.Goddard, Department of Cellular and Molecular Biology, AFRC Institute of Animal Physiology and Genetics, Edinburgh Research Station, Roslin, Midlothian, EH25 9PS, UK.

The manipulation of avian growth is scientifically and commercially important. Progress has been made in improvement of growth rate by genetic selection but little is known about the biological basis of the genetic control of growth. It is not clear which genes are the most appropriate to manipulate in order to affect this important trait. Before transgenic biology can be used to improve efficiency of growth in poultry, the regulatory factors must be identified, the genes encoding them cloned, and regulation of their expression clearly defined. The somatotrophic axis is involved in the post-hatch growth of the chicken and we are investigating the regulation of some of the genes concerned. The introduction of the chicken growth hormone (GH) gene into the avian genome and the effects of recombinant DNA-derived chicken GH on growth rate have been reported but in general results have been disappointing, perhaps due to a low expression of the GH receptor gene. We have isolated a number of GH receptor clones from a chicken liver cDNA library using a 650 bp rabbit GH receptor cDNA which spans the transmembrane domain and includes both intra- and extracellular domain sequences. These are currently being sequenced. The effects of GH are mediated by insulin-like growth factor-I (IGF-I) and a number of chicken IGF-I cDNA clones have been isolated. One of the clones is homologous to exons 1, 2, 3 and 5 of human IGF-I cDNA with another exon (1A) spliced between exons 1 and 2. A second clone contained sequence homologous to exons 1 and 2 with no intervening exon 1A, indicating at least two forms of IGF-I mRNA. IGF-I mRNA was detected in a number of tissues from rapidly growing broilers by reverse transcriptase/PCR using oligonucleotide primers derived from exons 2 and 3 of the chicken IGF-I sequence. The expression of the IGF type I receptor in hepatic membranes and myogenic cells has also been examined. Binding studies and affinity cross-linking experiments demonstrated a single type of IGF receptor in chick tissues. The receptor was developmentally regulated in the liver. Receptor number increased between 1 and 7 days of age and then decreased again by 21 days of age. Potential chicken IGF type I receptor clones isolated by hybridisation to the human IGF type I receptor are currently being sequenced and initial data shows up to 90% homology at the nucleotide level in the α-subunit domain. Myogenic cells derived from fast- or slow-growing chickens have different rates of proliferation in response to growth factors present in serum when seeded at the same density and cultured under identical conditions. This emphasises the importance of receptors, and the analysis of GH and IGF-I receptor gene expression using these cloned cDNAs may explain these observations.

CI 013 THE MAJOR HISTOCOMPATIBILITY COMPLEX IN CHICKENS. S. J. Lamont, Department of Animal Science, Poultry Section, Iowa State University, Ames, Iowa 50011.

The major histocompatibility complex (MHC) genes encode highly polymorphic cell-surface proteins of three classes. These antigens are markers of "self" and are important in regulation of cellular communication in the immune response. Restriction of cellular interactions by the MHC includes such phenomena as T-B cell cooperation in antibody production, generation of germinal centers, antigen presentation do T cells and T cell cytotoxic reactions against virally infected cells. Via its role as a restriction element or via specific immune response genes, the MHC has profound effects on genetic control of immunoresponsiveness. The chicken MHC is associated with total immunoglobulin levels, antibodies to synthetic peptides, soluble antigens, and cellular antigens, cell-mediated immunity, total hemolytic complement, chemotaxis and activity of macrophages and the percentage of T cells of specific subsets. The MHC has also been associated with various production traits such as mortality, egg production, body weight and feed efficiency.

The B-G antigens (class IV) are expressed on erythrocytes and other cell types. Although an immune regulatory function is unknown, the B-G antigens are highly immunogenic and appear to be necessary as an adjuvant for anti-B-F response. The B-F antigens (class I), expressed on all cell types, consists of 39-43 kDa glycoproteins of 3 extracellular domains and are associated with $\beta 2$ microglobulin. The B-L (class II) antigens consist of one nonpolymorphic α chain associating with polymorphic β chains and are primarily expressed on cells of the immune system. Recent molecular cloning of B complex genes has helped establish a molecular map of the chicken MHC. Although it seems that the overall exon/intron structure, domain organization and tertiary structure in similar to that of their mammalian counterpart, there are several striking differences. The B-F and B-Lβ genes are intermingled rather than in separate class I and II regions, there is no evidence for an intervening class II region, and the intron size is greatly reduced. The telomeric end of the MHC is adjacent to the nucleolar organizing region. There are several non-MHC genes interspersed in the region, including Gene C8.4 which has characteristics of the immunoglobulin super family and Gene C12.3, which bears homology to GTP-binding proteins.

The important role of the chicken MHC in health and fitness as well as rapid advances in molecular definition, makes it a good candidate for a genetic marker in breeding populations and also for genetic engineering.

Banquet Address

CI 014 POULTRY BREEDING TECHNOLOGIES IN THE 20TH CENTURY: WHERE HAVE WE COME FROM AND WHERE ARE WE GOING? R.N. SHOFFNER, PROF. EMERITUS, UNIVERSITY OF MINNESOTA, DEPARTMENT OF ANIMAL SCIENCE, ST. PAUL, MN 55108

There have been distinct times of enthusiasm in poultry breeding and genetics. The "show" period saw the development of many breeds and varieties. The application of classical genetics revealed much about the inheritance of morphological traits and beginning of gene mapping. The theory and practice of quantitative genetics technology resulted in considerable improvement in performance, with a dramatic change from many small breeding units and general farm producers to a few breeding companies and exceedingly large production units. Cytogenetic technology developed chromosome karyotypes, determined number and other pertinent knowledge about chicken chromosomes. Immunogenetic research determined the Ea-blood groups and the major histocompatibility complex (MHC) inheritance. Molecular genetic technology has generated enthusiasm as a tool for understanding basic biology and for aid in genetic change. Chromosome mapping, gene location and genomic identification will be advanced by use of restriction enzyme polymorphism (RFLP), finger printing, sequence-tagged sites (STS), DNA-DNA hybridization and other techniques. The prospect of gene transfer as a tool for inducing genetic variability is exciting. Plasmids, liposomes, and retroviral vectors, combined with novel means of stem cell insertion, will result in successful transfers. A major bottleneck is identification of "useful" genes because of limited knowledge about the basic biology of the fowl. The information presented at this symposium is intended to remedy this situation.

Current and Potential Applications of Molecular Genetics

CI 015 GENETIC CONTROL OF DISEASE AND DISEASE RESISTANCE IN POULTRY, Jan S. Gavora, Animal Research Centre, Agriculture Canada, Ottawa, Ontario, Canada K1A 0C6

Avian diseases in the broad sense include health problems arising from adverse physical environment or inadequate nutrition, developmental disorders, and infectious and parasitic diseases. Genetic improvement is of primary importance in the reduction of losses from the latter two groups of diseases. There is considerable knowledge of genetic mechanisms underlying many developmental disorders, mainly where major genes are involved, but their molecular basis is largely unknown. Ample evidence exists that genetic factors play an important role in resistance to infectious diseases, but only in a few instances is there evidence of major genes underlying the resistance. Knowledge of molecular bases of resistance mechanisms, such as the major histocompatibility system, immune response, and pathogen receptors is rapidly increasing. Also, the molecular structure of many pathogen genomes is known and can be used in devising approaches to the improvement of host resistance. Molecular genetic information may provide tools for genetic improvement of resistance by selection using markers that are either linked to or are themselves resistance genes. Such selection does not require exposure to pathogens and, therefore, is desirable from the animal welfare point of view. As well, DNA fingerprinting can improve the efficiency of transfer of genetic resistance by crossbreeding and backcrossing. Molecular gene transfer is applicable and justified primarily to introduce new resistance mechanisms, exemplified by pathogen-mediated resistance or antisense RNA, that do not exist in the avian species. Improvements of existing resistance mechanisms by gene transfer should await perfection of methods, such as embryonic stem cells and homologous recombination. Progress in molecular mapping of avian genomes and further elucidation of molecular bases of resistance will play an important role in future improvements of genetic resistance to diseases in poultry.

CI 016 USING MOLECULAR MARKERS IN POULTRY BREEDING AND PRODUCTION

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Recently developed methodologies may lead to the identification of DNA markers linked to loci affecting quantitative traits. The detection of such linkage requires the saturation of the genome by hundreds of markers and data derived from many progeny (1). Lately, it has been shown that the use of progeny from the extremes of a family distribution, may increase the chance of detecting linkage between markers from a previously mapped genome and QTLs (2). This selective genotyping, may detect even small QTL effects and decreased the required family size significantly. The fine and accurate mapping of QTLs has been significantly advanced following the development of QTL likelihood mapping using a complete RFLP map and new analytical methods (3). The most prominent contribution was the increased availability of minisatellite and microsatellite DNA probes each of which detects numerous polymorphic loci in poultry (4,5). A novel approach, waiving the need for a detailed genetic map and leading to the identification of DNA markers linked to QTLs, applies multi-locus DNA probes to DNA mixes (6) from individuals situated at the two extreme tails of progeny distribution (Tail Analysis). This method followed by linear model (LM) analyses was applied to the analysis of abdominal fat deposition in broilers (7). Each paternal-specific DNA fingerprint band was analyzed as a dummy independent variable and the quantitative trait value of 69 paternal half sibs was analyzed as the dependent variable. A band (S6.1), strongly associated with abdominal fat percentage was identified. The band effect estimated by LM was found to be approximately 30% of the family mean of the abdominal fat percentage.Progeny tests were used to validate this association presumably resulted from a linkage between band S6.1 and a QTL or a cluster of QTLs affecting abdominal fat deposition. The LM analysis was applied to seven additional quantitative traits in this family. In six of them, highly statistically significant associations between DNA fingerprint bands and performance were observed. One of the major advantages of this methodology is its complete independence of a genetic map.

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CI 017 SEX-SPECIFIC DNA SEQUENCES IN THE BIRD AND THEIR APPLICATION TO THE STUDY OF SEX DETERMINATION, Shigeki Mizuno, Laboratory of Biochemistry, Department of Agricultural Chemistry, Tohoku University, Sendai 981, Japan

Four different W chromosome-specific repeating DNA units were cloned and sequenced from birds belonging to the order Galliformes: 0.7kb XhoI(1) and 1.2kb EcoRI fragments from chicken, 0.4kb PstI fragment from turkey(2) and 0.5kb TaqI fragment from pheasant(2). Approximate repetition frequencies of these units in the diploid genomes are: 14,000(XhoI-0.7kb), 700 to 4,000(EcoRI-1.2kb). 10,000(PstI-0.4kb) and 4,000 times(TaqI-0.5kb). In the female chicken, XhoI and EcoRI families, including highly related sequences to the above repeating units, account for about 60% and 10-30%, respectively, of the DNA in the W chromosome. Although overall sequence similarities among the four repeating units are 60 to 70% they share common structural features that they all consist of tandem repeats of about 21bp units, most of which contain (A)3-5 and (T)3-5 clusters separated by 6-7 nucleotides and that they all behave as remarkably bent molucules in solution.

Sex of an individual chicken embryo at as early as 48h-incubation could be determined unambiguously by slot-blot hybridization of DNA extracted from the extra-embryonic area with the digoxigenin-labeled 0.7kb XhoI fragment followed by reaction with an alkaline phosphatase-linked anti-digoxygenin antibody. We have investigated time of expression of two genes involved in the biosynthesis of sex steroid hormones; i.e. genes of cytochrome P-450c17(3) and aromatase, applying the PCR cDNA amplification procedure to the total RNAs extracted from male and female embryos at different developmental stages. These experiments demonstrated that the P-450c17 mRNA was already present in the 72h embryos of both sexes but appreciable amount of the aromatase mRNA was first detectable in the 7 day female embryos. These results imply that onset of transcription of genes in the pathway of sex steroid synthesis is not coordinated and may be categorized into groups, one starting before the differentiation and one starting after or during the differentiation of gonads.

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Opportunities and Constraints in the Manipulation of the Avian Genome from an Economic and Scientific Perspective

CI 018 *ASPIRATIONS OF BREEDERS IN THE POULTRY INDUSTRY AS MANIPULATION OF THE AVIAN GENOME CONTINUES,* *Alan Emsley, ISA Babcock, P.O. Box 280, Ithaca, NY 14851-0280*

The Poultry Breeding industry has been very receptive to new technology. Future commercial breeding methods will undoubtedly be influenced by the rapid advances in molecular genetics research, but it is not at all clear which of today's research efforts hold the greatest promise.

For any new means of genetic manipulation to merit incorporation into present methods, it must offer to the breeder the possibility of greater competitiveness in a multifaceted marketplace. Breeding objectives are comprehensive and sculpt the selection programs which seek to achieve balanced genetic gain. As demonstrated by this symposium, new developments in methods of genomic manipulation offer a palette of options from which each commercial breeder may select. The most attractive choices will be those with clear definition about the potential impact on total performance and the time required to see that improvement manifested in the field. Simultaneous adoption of the same technological advances by all breeders is very unlikely. Thus, temporary advantages are possible for those who can discern correctly what is useful to their own needs and utilize it promptly. Ultimately, the consumer is the beneficiary of enhancements in overall productivity.

The greatest and most ambitious hope is that new technology will, at relatively low cost, provide a means of improving the rate of genetic gain in commercial cross performance through estimation of breeding value earlier in life than is now possible, with greater accuracy and with equal ease from both sexes.

On the other hand, a simple risk-free method of sex control could radically alter the efficiency of operation at the hatchery level.

Current breeding methods, despite their continuing success, face the risk of declining marginal rates of genetic improvement in multi-trait performance because of fixation of important alleles, increased relative importance of non-additivity, a shift in priority toward traits measured later in performance, and greater genetic antagonism among constituent traits and between pureline and crossline performance. Enhancement of current programs by novel breeding technology which would mitigate these trends would be welcomed.

Methods of genetic manipulation offer hope for refinement of our genetic model, and possibly our optimum breeding strategy, through greater understanding of basic biology underlying phenomena such as intralocus and interlocus genetic interactions, controls over each tissue's developmental agenda, age effects on performance, nutrient utilization, disease resistance and inbreeding depression.

CI 019 FUTURE INTERACTIONS BETWEEN MOLECULAR BIOLOGISTS AND THE POULTRY INDUSTRY, Ann M. Verrinder Gibbins, Department of Animal and Poultry Science, University of Guelph, Guelph, Ontario, Canada, N1G 2W1.

There is no doubt that our knowledge of avian molecular genetics, cell biology and embryology will develop markedly during the next ten years. Whether or not this expansion in knowledge will be as extensive and as effectively applied as could be possible will depend on the way in which researchers, industry and government interact in the future. To be successful, industry must develop a competitive edge and accumulate profit, often through the exploitation of new developments in avian biology. Avian biologists develop new ideas with financial support from industry and government, preferably in a climate that encourages interchange of ideas and with freedom to conduct basic research that provides no obvious gain in the short term, but may prove to be crucially relevant to an applied project in the long term. There is a danger that the necessary contractual arrangements between members of the poultry industry and researchers may impede interchange of ideas between biologists to the extent that the rate of development of knowledge in avian biology will be impaired. A strategy will be presented for the promotion of regular communication between avian biologists in research laboratories and industry in order to develop a more productive type of community spirit. Such interaction will be essential for the rapid and efficient implementation of major programmes such as the development and use of chicken and turkey genome maps or the development of procedures for the construction of "transgenic" birds with altered gene expression.

Poster Session

CI 100 **THE CHICKEN GENOME CONTAINS MANY ev-GENE LOCI,** *Henk J.M. Aarts, Ria C. van der Hulst, Gerard Beuving and Ferry R. Leenstra, "Het Spelderholt", Centre for Poultry Research and Information Services, Spelderholt 9, 7361 DA Beekbergen, The Netherlands.*

Endogenous viral genes (ev-genes), which are closely related to the Avian Leukosis Virus (ALV), are randomly distributed throughout the chicken genome. The number of these ev-genes varies significantly: e.g. in White Leghorns on average 2 ev-genes/individual were found and in Cornish type chickens 5-6 ev-genes/individual. Extensive variation was also found in the ev-gene patterns among animals of the same type or line. The identification of ev-genes is very difficult especially in birds posessing a lot of ev-genes. By comparing the BamHI and SstI ev-gene hybridization patterns of the offspring of crosses between 20 birds having complex ev-gene patterns and ev-0 birds we could identify at least 35 different ev-genes. The differences observed among animals of the same type or line are mainly caused by a high number of ev-gene loci and that within one bird only a few of these loci are occupied. This results in a high degree of heterozygozity for the 'visible' ev-gene loci (>80%). Our experiments show that the investigated ev-gene loci are not multiple allelic. Ev-genes in different types of chickens differ in RFLP pattern and highly probable occupy different loci.

CI 101 AFFINITY PURIFICATION AND CHARACTERIZATION OF TURKEY GONADOTROPIN
K.D. Taylor, C.E. Anderson-Langmuir and R.J. Etches, Dept. of Animal and Poultry Science, University of Guelph, Guelph, Ontario, Canada. Avian gonadotropins have not been well characterized and the polyclonal antibodies which have been raised to them have shown cross reactivity to avian TSH. These problems have been addressed by raising monoclonal antibodies designated MCA B51.5 and C41.1 against turkey luteinizing hormone (tLH B221B) and Con A-Sepharose purified turkey pituitary homogenate designated MCA T13.3. Protein A purified monoclonal antibodies were bound to hydrazide activated agarose gel (Affi-gel HZ, Bio Rad) and used to isolate gonadotropins from turkey pituitary homogenate. Characterization of the affinity purified gonadotropins using SDS PAGE has shown that they contain a double band corresponding to the 14.4kD standard. Western blotting analysis has revealed that the monoclonal antibodies and a polyclonal anti-chicken LH antibody (M201) will bind to the affinity purified gonadotropins. Relative bioactivity of the affinity purified gonadotropins was determined using chicken granulosa cell, Japanese Quail interstitial cell and chicken ovulation inducing assays. All of the affinity purified gonadotropins have significant biological activity in the steroidogenic assays and approximately 1/100 of the activity of NIAMMD bLH-5 in the ovulation inducing assay. These data indicate that ovulation in the hen may require more than one gonadotropin molecule.

CI 102 INTRODUCTION OF LIPOFECTED CHICKEN BLASTODERMAL CELLS INTO THE EARLY CHICKEN EMBRYO, Cynthia L. Brazolot, James N. Petitte*, Mary E. Clark, Robert J. Etches and Ann M. Verrinder Gibbins, Department of Animal & Poultry Science, University of Guelph, Guelph, Ontario N1G 2W1, and *Department of Poultry Science, North Carolina State University, Raleigh, NC 27695.
We have shown previously, by using phenotypic markers and DNA fingerprinting, that chicken blastodermal cells (CBCs) removed from the unincubated embryo can be transferred to recipient stage X embryos and will contribute to the germline, melanocytes and hematopoietic tissues of resulting chimeric birds (Petitte *et al.*, Development *108*:185-189, 1990); the donor CBCs can be transfected efficiently (at least one expressing cell out of every 25 cells treated) using Lipofectin™ (Brazolot *et al.*, Cell Differentiation and Development *27*, Supplement 1, S90, 1989). CBCs were lipofected with constructs containing bacterial *lacZ* reporter gene sequences under the control of an apparently constitutive chicken β-actin/RSV promoter, a Zn^{2+}-inducible chicken metallothionein (cMt) promoter, or an apparently constitutive CMV-driven promoter; in the latter case, the β-galactosidase was located in the nucleus. Following transfer of transfected CBCs into recipient embryos and incubation for 48-65 hours, the embryos were stained for β-galactosidase activity either immediately or after induction of the cMt promoter using 150 μM $ZnCl_2$. Bacterial β-galactosidase-expressing cells, individually and in foci, were observed in embryonic tissues following transfer of donor cells lipofected with the β-actin/RSV promoter-driven *lacZ* construct, and in extraembryonic tissues following transfer of cells containing any of the three gene constructs. Transfer of lipofected CBCs into recipient embryos is therefore a feasible route for the production of transgenic chickens.

CI 103 A GENE TRANSFER VECTOR FOR POULTRY WHICH EXPRESSES MOUSE TYROSINASE, J. Brumbaugh, T. Frew, B. Whitaker. Biological Sciences, U of Nebraska, Lincoln, NE 68588, USA; H. Yamamoto, T. Takeuchi. Biological Sciences, Tohoku U, Sendai 980, Japan; S. Hughes. Frederick Cancer Research Facility, Frederick, MD 21701, USA; D. Salter, W. Payne. Depts Animal Science and Microbiology, Michigan State U and RPRL, E Lansing, MI 48823, USA

A cDNA encoding mouse tyrosinase was inserted into a plasmid containing the provirus of a replication competent avian leukosis virus. A viral stock produced from the plasmid was used to infect cultured cells and developing chick embryos. Cultured melanocytes of the autosomal albino genotype were ostensibly cured by the expressing mouse tyrosinase gene and produced melanosomes. However, the sex-linked albino and White Leghorn genotypes, which produce some pigment in culture, showed no overt increase in melanin synthesis. On the other hand, cultured fibroblasts and liver cells of the White Leghorn genotype produced gray to black pigment. White Leghorn chick embryos injected with virus on days 0-3 of incubation produced chicks which have decidedly red-orange down as opposed to the white down of the controls. Some of these chicks on autopsy at 3-4 weeks were expressing tyrosinase in their livers as evidenced by numerous black lesions. These results indicate that tyrosinase is being expressed constitutively, as expected, from the viral promoter. Tyrosinase gene expression in chicks suggests that with tissue specific promoters this type of vector will become a useful marker for trangenic studies.

CI 104 DEVELOPMENTAL PROFILE OF GROWTH HORMONE RECEPTOR GENE EXPRESSION IN BROILER CHICKENS, Joan Burnside and Larry A. Cogburn, Department of Animal Science and Agricultural Biochemistry, University of Delaware, Newark, DE 19717

In the growing animal, growth hormone (GH) is the primary somatogenic hormone and promotes cell growth indirectly by stimulating hepatic synthesis of IGF-I or directly by interacting with other tissues. GH activity is mediated and may be limited by interaction with plasma membrane bound receptors. GH gene expression is developmentally regulated; serum GH levels increase posthatching, reach a maximum at 3-4 weeks, and thereafter sharply decline. In order to better understand the parameters and limiting factors involved in GH signal transmission, we have examined the ontogeny of hepatic GH receptor expression from day 13 embryonation to 31 weeks after hatch. A cDNA probe for the chicken GH receptor was used in Northern blot analysis of total RNA. Three species of mRNA transcripts are detectable, possibly representing two different forms of the receptor and the serum GH binding protein. Expression of the receptor is very low, although detectable, in embryonic liver. Embryonic growth is generally thought to be GH independent; the presence of GH receptors suggests that GH may function in early development. The expression of the GH receptor is markedly enhanced in the adult chicken, gradually increasing to a maximum level between 19 and 31 weeks of age. Thus, the GH receptor may play a key role during embryonic development as well as performing important endocrine functions in the adult. The lack of synchronous expression between GH synthesis and secretion and GH receptor expression is of interest. The GH response in young birds may be limited by the amount of GH receptor.

CI 105 GRADIENTS OF HOMEOPROTEINS IN DEVELOPING FEATHER BUDS, Cheng-Ming Chuong, Guillermo Oliver, Sheree A. Ting, Beatrice G. Jegalian, Hai Ming Chen and Eddy M. De Robertis, Department of Pathology, University of Southern California, Los Angeles, CA 90033 and Department of Biological Chemistry, UCLA, Los Angeles, CA 90024.

Homeoproteins are functionally involved in pattern formation. Here we examine the expression of homeoproteins in chicken feather development by immunocytochemical localization. We find that XIHbox 1 antigen is present in cell nuclei and is distributed in a gradient in the mesoderm of developing feather buds, with strongest expression in the anterior-proximal region. The gradient is most obvious in feather buds from the mid-trunk level. Feather buds from the scapular level express very high levels of XIHbox 1 and feather buds from the caudal region express no XIHbox 1, suggesting that a broad gradient along the body axis is superimposed on a smaller gradient with each individual feather bud. Another homeoprotein, Hox 5.2, is also expressed in developing feather buds in a graded way, and its distribution pattern is partially complementary to that of XIHbox 1. These observations suggest that homeoproteins may be involved in setting up the anteroposterior polarity of cell fields at different levels, first for the body axis, then for the limb axis and finally for the feather axis.

CI 106 THE EFFECTS OF A DEFICIENCY IN rRNA GENE COPY NUMBER ON PROGRESSION OF THE EARLY CHICK EMBRYO THROUGH DEVELOPMENT, Mary E. Delany and Stephen E. Bloom, Department of Poultry and Avian Sciences, Cornell University, Ithaca, NY, 14853.
--We are using a genetic approach to study development in the chicken by investigating the effects of deficiencies for rRNA gene copy number on embryonic development. In the chicken, *ca.* 300 copies of the rRNA genes are organized on one chromosome pair. Previously, we have shown by quantitative *in situ* hybridization that nucleolar size polymorphisms reflect heterozygosity for rRNA gene copy number. That is, a macronucleolus is organized by a rRNA gene set larger than that which organizes a micronucleolus. Crosses between chickens heterozygous for rRNA gene copy number (1 macro- and 1 micronucleolus/cell, **Pp**) should result in a 1:2:1 ratio of 3 progeny types (**PP**:**Pp**:**pp**) if all classes are equally viable. Embryos were recovered from unincubated eggs, and from eggs after 24, 36, 48, 60, and 96 hrs of incubation. **PP** and **Pp** progeny types were recovered at all time points, whereas **pp** embryos (exhibiting aberrant nucleolar morphology) were recovered only from unincubated eggs, and eggs incubated for 24 and 36 hrs; the recovery of **pp** individuals decreased as developmental time progressed. Early embryonic development is dependent on the oocyte provision of ribosomes whose rate of depletion must be compatible with the initiation of transcription of embryonic rRNA at a rate sufficient for massive production of ribosomes. Our results show that although rRNA synthesis and nucleolus formation begin during mid-cleavage in the chicken (Wylie 1972, Raveh *et al.* 1976), the stores of maternal ribosomes are large enough to carry an embryo deficient in rRNA gene copy number through gastrulation. (supported by grants from the NIEHS ESO3499 and USDA NY157433)

CI 107 MAPPING OF SINGLE COPY GENES TO CHICKEN CHROMOSOMES USING IN SITU HYBRIDIZATION, Marina Dominguez-Steglich and Michael Schmid, Dept. of Human Genetics, University of Würzburg, Federal Republic of Germany.

Use of chemically and/or radioactively modified DNA probes binding in situ to fixed target DNA can determine the exact chromosomal localization of specific sequences. Gene mapping may be greatly assisted by employing this technique. In situ hybridization of a tritium-labeled cDNA probe corresponding to the ornithin transcarbamilase gene (OTC) shows a peak of silver grain accumulation on the Z chromosome. This would be the first example of an X-linked gene in mammals (XX/XY system of sex determination) also being sex-linked in birds (ZZ/ZW system). Closely linked to OTC on the mammalian X chromosome is the huge and highly conserved gene coding for dystrophin. In situ hybridization using a pool of biotinylated cDNA probes covering the locus allowed detection of this single-copy sequence without needing isotopic labeling. It was found to be autosomal in the chicken, mapping to a pair of microchromosomes. Non-radioactive in situ hybridization was also successful in confirming linkage of the chicken B-complex to the nucleolus organizer regions.

CI 108 COMPARISON OF LIPOGENIC ENZYMES AND APOPROTEINS MESSENGER RNA FROM GENETICALLY LEAN AND FAT CHICKENS, Madeleine Douaire, Patrick Langlois, Nathalie Le Fur, Fréderic Flamant, Catherine Mounier, Jacques Mallard, Laboratory of animal genetics, Institut National de la Recherche Agronomique, 35042 Rennes Cedex, France
Quantitative traits are usually considered to be under the control of many interacting genes. However, in the case of poultry fatness, both the knowledge of metabolic pathways and the data of genetic selection suggest that only few genes are responsible for the observed variability. We analysed liver mRNA for lipogenic enzymes (fatty acid synthase, acetyl-CoA carboxylase, malic enzyme) and apoproteins (apoB, apoA1, apoVLDL II) coding genes in genetically lean and fat 9 weeks old male chickens. Comparisons were performed to assess if genes expression levels could be related to abdominal fat pad. Any correlation could be directly used to screen breeding populations for favorable genotypes.

CI 109 ULTRASTRUCTURAL MORPHOLOGY OF THE PRE-PRIMITIVE STREAK CHICK EMBRYO.

J.M. Watt, J.N. Petitte* and R.J. Etches. Department of Animal and Poultry Science, University of Guelph, Guelph, Ontario and *Department of Poultry Science, North Carolina State University, Raleigh, N.C.

A successful method for the production of somatic and germline chimaeras has been developed in the chicken (Petitte *et al*, 1990. Development **108**, 185). The technique involves the transfer of cells from stages V-X (Eyal-Giladi and Kochav, 1976. Dev Biol **49**, 321) into recipient stage X embryos. Using the techniques of scanning and transmission electron microscopy, our aim has been to discover which of the aforementioned stages is optimal, in terms of morphological differentiation, for transferring into recipient embryos in order that donor cells become incorporated. Accordingly we have examined embryos at stages IV-XIV using SEM and TEM. At stage IV, a subgerminal cavity is present although some yolk remains in close apposition to the blastodermal cells. By stage V a distinct fluid-filled subgerminal cavity delineates the blastodermal cells from the underlying yolk. During stages VI-IX there is little change in cellular appearance and the epiblast is composed of 3 cell layers. At stage X the uppermost layer of epiblast is epithelial-like and the subepithelial layers number two to three. Stage XI embryos are markedly different from previous stages in that cells of the secondary hypoblast display long filopodia and ruffled lamellipodia in preparation for migration along the flat meshwork of cells provided by the stellate cells of the underside of the epiblast. During stages XII-XIV cells in the posterior hypoblast, marginal zone and area opaca assume distinct morphologies in each area.

CI 110 **AVIAN RETROVIRUS VECTORS WITH INTERNAL PROMOTER. INFLUENCE OF 3' NON CODING SEQUENCES ON GENE TRANSFER SAFETY AND EFFICIENCY.** Frédéric FLAMANT, Denise AUBERT, Jacques SAMARUT Laboratoire de différentiation et Oncogénèse virale. Ecole Normale Supérieure. Lyon. France.

It has been shown that a mutation in the 3' LTR of a retrovirus can lead to the inactivation of the 5' LTR transcription promoter after one round of replication. This self-inactivation process enables to improve the safety of retrovirus mediated gene transfer and favours the activity of transcription promoters located within a retrovirus vector (Cone et al. 1987, Mol. Cell. Biol. 7, p887).We have constructed several vectors to test the possibility to derive self-inactivating vectors from avian leukosis viruses. All these vectors carry (from 5' to 3'), a fully active RSV LTR, the NeoR selection gene and the nls-lacZ gene driven by an internal SV40 promoter , but differ by their 3' non coding sequences. These constructs were transfected into the Isolde avian helper cell line and supernatants harvested from stably transfected cells were titrated on QT6 cells. An *in situ* assay reveals the transfer of the internal SV-nlslacZ gene (titre in lac+fu/ml), while G418 resistance resulted from the NeoR gene expression driven by the 5' LTR.

When a fully active 3' LTR was used, high virus titre was obtained in both assays, confirming that avian vectors can accomodate an internal promoter. From a construct carrying a deleted RAV(0) 3' LTR, we were able to select a helper cell clone giving promising results for self-inactivation (1000 lac+fu/ml and 60 G418rfu/ml). Further analysis demonstrated that 3' non-coding sequences influence not only the self-inactivation ability of the vector but also the internal SV-nlslacZ gene expression, the RNA packaging and probably other steps in the retrovirus life cycle. The ability of these vectors to transfer a gene driven by its own promoter *in vivo* with improved safety is currently tested.

CI 111 THE ACCESSORY REPRODUCTIVE FLUIDS IN THE DOMESTIC MALE BIRDS,

Fujihara Noboru and Koga Osamu, Department of Animal Science, Kyushu University 46-06, Hakozaki, Fukuoka 812 Japan

Roosters and male turkeys ejected a lymph-like fluid and a foam at the time of ejaculation, and drakes produced a lymph-like fluid without containing foam. Male quail produced a meringue-like foam, quite different from the above-mentioned fluid and foam. On the other hand, guinea fowls and pigeons produced neither lymph-like fluid nor foam during ejaculation. The lymph-like fluid was ejected from the ventral cloacal tissue, and the foam was secreted by the dorsal cloacal gland. The former was blood plasma-originated fluid like lymph, and the latter was mucopolysaccharide secreted by the glandular tissue. The lymph-like fluid stimulated sperm motility, and improved fertilizing ability of spermatozoa shortly after ejaculation, but the meringue-like foam of the quail was not effective for enhancing sperm motility and fertilizing capacity. In contrast, the lymph-like fluid and foam were detrimental to spermatozoa when semen mixed with the fluid were preserved in vitro, resulting in the loss of sperm fertilizing capacity due to an increased number of deformed spermatozoa.

CI 112 AVIAN CELLS EXPRESSING THE MURINE Mx1 PROTEIN ARE RESISTANT TO INFLUENZA VIRUS INFECTION; Ellen A. Garber[1], Hilary T. Chute[1], Jon H. Condra[2], Leah Gotlib[2], Richard J. Colonno[2], Edward O. Mills[3], Jane Hancock[3], Donna Hreniuk[1], and Roy G. Smith[1], [1]Dept. of Animal Biochemistry and Molecular Biology, Merck, Sharp, and Dohme Research Laboratories, Rahway, NJ 07065; [2]Dept. of Virus and Cell Biology, Merck, Sharp, and Dohme Research Laboratories, West Point, PA 19486; [3]Hubbard Farms, Walpole, NH 03608.

The cDNA encoding the murine Mx1 protein, a mediator of resistance to influenza virus, was inserted into a replication-competent avian retroviral vector in either the sense (Mx^+) or the antisense (Mx^-) orientation relative to the viral structural genes. Both vectors produced virus retaining the Mx insert following transfection into chicken embryo fibroblasts (CEF). Mx protein of the appropriate size and nuclear localization was expressed only in CEF cells infected with the Mx^+ virus. Mx expression was observed in all Mx^+ virus-infected cells and was stable during long-term culture. Cells infected with the Mx^+ virus were resistant to infection by human influenza A/WSN/33 (H1N1) and avian influenza viruses A/Turkey/Wisconsin/68 (H5N9) and A/Turkey/Massachusetts/65 (H6N2), but were susceptible to infection by the enveloped RNA viruses Sindbis and vesicular stomatitis virus (VSV). Normal CEF and cells infected with the Mx^- virus were susceptible to influenza A, Sindbis, and VSV. The synthesis of influenza proteins, especially the larger polymerase and hemagglutinin proteins, was reduced in Mx^+ retrovirus-infected cells superinfected by influenza A.

Chickens infected *in ovo* on day 1 with the Mx^+ retrovirus were hatched. Infection with the virus did not compromise development and hatchability. Mx expression was detected in blood cells from viremic chickens.

CI 113 THE INTERSPECIFIC TRANSFER OF AVIAN PRIMORDIAL GERM CELLS, Teresa C. Guido, Ursula K. Abbott, Department of Avian Sciences, University of California, Davis, CA 95616, John R. McCarrey, John Hopkins University, School of Hygiene and Public Health, Baltimore, MD 21205

Avian primordial germ cells (PGCs) move into the blood islets developing in the anterior germinal crescent of the early embryo and then through the circulatory system to the germinal ridge area to become incorporated subsequently into the presumptive gonads.

We removed gonads from Japanese quail incubated for 6 days (st. 29-30 H&H), enriched the PGCs to a concentration of 30-50% using a mini STA-PUT gradient system, and then injected them into the vitelline vein of embryonic chicken hosts at 3 1/2 days of incubation (st. 19-20 H&H). These host embyros were incubated another 6 days and then sacrificed at 10 days of incubation (st. 34-35 H&H), and their gonads removed, fixed, embedded and sectioned. Putative chimeric gonad sections were Feulgen-stained, so that donor Japanese quail PGCs could be distinguished from those of the host by the intense staining of the two or more nucleoli present.

This study enabled us to observe that the older transplanted Japanese quail PGCs developed at a rate reflecting the developmental age of the donor rather than that of the developmentally younger host. This finding suggests that the rate of development of embryonic germ cells is regulated intrinsically. We are exploring the use of this system to introduce exogenous genetic information into the avian germ line.

CI 114 ATTEMPTS AT PRODUCTION OF TRANSGENIC CHICKENS BY MANIPULATED PRIMORDIAL GERM CELL. Jae-Yong Han, Kevin S. Guise, and Robert N. Shoffner, Department of Animal Science, University of Minnesota, St. Paul, MN 55108.

Genetically manipulated primordial germ cells (PGC) might be used to introduce foreign DNA into the germline. As a goal of genetic modification is to ultimately control the genetic material of the germline, the modified primordial germ cell is the best vector for introduction of DNA into germline. Preliminary experiments were conducted to study the transfection efficiency of cultured chicken fibroblasts by four different methods *in vitro*. Calcium Phosphate mediated transfection, Lipofectin mediated transfection , DEAE-Dextran transfection and polybrene / DMSO methods were compared by transfection of plasmid RSVLTR / ß-gal 2. Calcium phosphate and Lipofectin mediated transfection showed high transfection efficiency (0.1% - 5%). In an attempt to transfect primordial germ cells in the early embryo, plasmid RSVLTR / ß-gal 2 DNA complexed with Calcium Phosphate and Lipofectin was injected into the blastoderm of unincubated and 24 hrs incubated fertilized embryos. The injected embryos were screened after 36 -60 hrs of incubation and 10-15% of infected embryos were positive transfection by RSVLTR / ß-gal 2 plasmid. Future experiments will involve transfection of PGC *in vitro* and *in vivo*. This includes the isolation of PGC, transfection of PGC *in vitro*, and microinjection of transfected PGC into germinal crescent area or vasculature of early embryo.

Cl 115 CULTURE OF THE PREOVULATORY AVIAN EMBRYO WITH CONSIDERATION OF CYROPRESERVATION, T.L. Hargrove*, G.F. Gee+ and M.A. Ottinger*, *Department of Poultry Science, University of Maryland, College Park, MD 20742 and +Patuxent Wildlife Research Centre, Laurel, MD 20708

Early development was studied in avian embryos obtained 4-6 hours post-ovulation. Prostaglandins were used to induce premature oviposition; embryos were typically at stage I-II, according to the staging of Eyal-Giladi and Kochav (Devel. Biol., 49, 321-337, 1976). Embryos were cultured in a submerged condition, using a modification of Perry's technique (Nature, 331, 70-72, 1988). Embryonic development was observed up to 48 hours of culture. In separate experiments, stage X embryos were dissociated and then frozen according to one of several protocols. Results indicated that greatest post-thaw viability was obtained using procedures that also have proven successful for freezing avian semen and mammalian embryos.

Cl 116 ISOLATION OF A DEVELOPMENTALLY REGULATED PROTEIN BINDING UPSTREAM OF THE APOVLDLII GENE FROM AVIAN LIVER, Pamela A. Hoodless and Roger G. Deeley, Cancer Research Laboratories, Queen's University, Kingston, Ontario, Canada.

Expression of the avian very low density apolipoprotein II (apoVLDLII) is estrogen dependent and restricted to the liver. We have examined protein binding sites in the 5' flanking region of this gene. One particular protein binding site, located 2.6kb upstream of the transcriptional initiation site, is of interest since it contains an Msp1 site which is demethylated between days 7 and 9 of embryogenesis. This corresponds to the developmental window when the gene can first be activated by exogenous estrogen. A single major protein/DNA complex is formed with liver nuclear salt extracts in gel mobility shift assays using oligomers corresponding to this site. Developmental studies in the liver indicate that the protein binding to this site is highest at day 7 of embryogenesis and levels decrease during development to levels found in adults. In addition, the protein/DNA complex undergoes a distinct increase in mobility between days 11 and 13 of embryogenesis. This site is structurally similar to sites that bind CCAAT/enhancer binding protein (C/EBP) and DBP in that it contains two inverted copies of the core enhancer sequence with only 1 mismatch at each copy. Competition studies show that this factor is not NF-1, HNF-1, C/EBP or DBP. In addition, developmental patterns of expression of this protein are inconsistent with those reported for C/EBP and DBP. We have purified the protein binding to this site from nuclear, salt extracts from laying hens approximately 3,000 fold using a combination of standard chromatography techniques and DNA affinity chromatography. Cross-linking studies indicate that the protein binding to this site is between 47kd and 55kd. Further characterization of this factor is in progress.

Cl 117 GENE MAPPING IN CHICKENS VIA FLUORESCENT IN SITU HYBRIDIZATION TO MITOTIC AND MEIOTIC CHROMOSOMES, Nancy J. Hutchison and Cosette LeCiel, Genetics Department, Fred Hutchinson Cancer Research Center, Seattle, WA 98104

The chicken genetic map (O'Brien, S.J., Genetic Maps, 1990) is suprisingly sparse for an animal with strong nutritional and economic importance. The mapped genes primarily represent physical traits, such as feather characteristics, while, with the exception of endogenous viruses and oncogenes, very few biochemically defined sequences have been mapped. Fluorescent in situ hybridization with biotin labelled probes allows us to map single copy and repetitive DNA sequences to mitotic metaphase chromosomes prepared from the chicken MSB-1 lymphocyte cell line. Using methods developed by Lawrence and Singer and Dave Ward's group, we are mapping genes with cDNA and genomic cloned probes. Ovalbumin and two closely linked genes, X and Y, were known to be on macrochromosome 1, 2, or 3. Our results indicate that this gene cluster maps near the centromere on the long arm of chromosome 2 (clones courtesy of Bert O'Malley, Houston, Tx). We find that the red blood cell specific histone H5 gene maps to the short arm of chromosome 1. The label from histone H5 is near the position labelled by a 4.3 kb EcoRI fragment specific to the 21 gene histone cluster. We find that co-hybridization with both the H5 and 4.3 kb probes shows two neighboring but separable sites of hybridization. Mammalian G bands are thought to contain 5-10 Mb of DNA; this provides a rough estimate for the distance separating the H5 gene from the large cluster of typical histone genes that we find on chromsome 1. (Histone clones provided by Julian Wells, R.D'Andrea, and P.Kreig.) Since we are particularly interested in the organization of the meiotic chromosomes we have also used chicken oocyte lambrush chromosomes and rooster spermatogenic spread preparations in these experiments. Hybridization with repetitive sequences from the W chromosome (clones provided by S.Mizuno) allowed us to identify the W at the lampbrush chromosome stage and show that it is present in a complex with the Z chromosome that looks like an end-to-end pairing or association. (Supported by NIH GM34873).

CI 118 PATTERN OF EXPRESSION OF TRANSFORMING GROWTH FACTOR-β4 IN THE DEVELOPING CHICKEN EMBRYO, Sonia B. Jakowlew, Jeremy Cubert, Michael B. Sporn and Anita B. Roberts, Laboratory of Chemoprevention, National Cancer Institute, Bethesda, MD 20892. cDNA probes and antibodies for TGF-β4 were used to study expression of TGF-β4 in the developing chicken. TGF-β4 mRNA was detected by day 4 of incubation and increased with developmental age until day 12. TGF-β4 mRNA was detected by RNA Northern blot analysis in every embryonic tissue examined, with expression being highest in smooth muscle and lowest in the kidney. At later stages of development, the level of expression of TGF-β4 mRNA remained elevated in neonatal and adult chicken striated muscle, smooth muscle and cardiac muscle, while it decreased in other tissues. Immunohistochemical staining techniques were used to detect TGF-β4 protein in many embryonic chicken tissues; significant expression was detected by the fourth day of incubation in specialized cells of the brain, heart, kidney and intestine, suggesting unique roles for TGF-β4 within the specific tissue. The level of TGF-β4 immunohistochemical staining decreased in most adult tissues except in spleen. Immunohistochemical staining of spleens of developing chickens showed increasing levels of immunoreactive TGF-β4 with increasing developmental age. Paralleling the increase in the level of TGF-β4 expression in spleen with age, the level of expression of TGF-β4 mRNA also increased significantly with developmental age in chicken spleen, with expression of TGF-β4 mRNA in the adult spleen being greater than that in the embryonic spleen.

CI 119 CHICKEN THY-1: cDNA ISOLATION AND mRNA EXPRESSION PATTERNS, Peter L. Jeffrey, Bruce J. Dowsing and Peter W. Gunning, Children's Medical Research Foundation, Camperdown N.S.W. 2050, Australia

A 23 kD glycoprotein has been identified in avian neuronal tissues which we have postulated to be the avian Thy-1 homologue (Sheppard et al., Dev. Brain Res. 40, 181-192; 43, 49-60 (1988)).

Confirmation that the 23 kD protein is the avian Thy-1 homologue was obtained from CNB and Endoproteinase Lys C peptides derived from protein purified on a Thy-1 specific monoclonal antibody affinity column of detergent extracted day old chick brains.

A combination of PCR, 3' RACE and screening of a ED19 forebrain pUEX-1 cDNA library has resulted in the isolation and sequencing of an 1847 bp Thy-1 cDNA clone encoding full protein sequence and 5' and 3' UTR's. Northern analysis of forebrain, cerebellum and tectum detect Thy-1 mRNA of 1.2 - 2 kb in size at ED5, 8 and 7 respectively, which show developmental increases. No signal is found in adrenal, bursa, heart, kidney, spleen, muscle and liver. Studies are underway, following Thy-1 expression at the various levels as well as surface localization.

CI 120 BACKTRANSPLANTATION OF CULTURED CARDIAC NEURAL CREST CELLS IN EARLY CHICK EMBRYOS RESCUES CARDIOVASCULAR DEVELOPMENT, Margaret L. Kirby, Donna H. Kumiski, Candace Rossignol, Department of Anatomy, Medical College of Georgia, Augusta, GA 30912-2000

Ablation of the premigratory cardiac neural crest in chick embryos results in a variety of intracardiac defects as well as anomalies of the derivatives of the aortic arch arteries. Replacement of the cardiac neural crest by other regions of the neural crest does not result in normal development of the cardiovascular system, indicating a specific role for the cardiac neural crest in cardiovascular development. The potential for alteration of single gene products in the cardiac neural crest is dependent on isolating those cells and replacing them in embryos prior to the time of cardiac neural crest migration. The neural folds were removed from the cardiac region of stage 9-10 quail embryos, plated in plastic dishes and cultured in DMEM with 10% chick embryo extract and 15% horse serum for 24 hours to two weeks. The neural fold was removed 24 to 36 hours after migration of the neural crest cells leaving an enriched population of cardiac neural crest cells. At various times, 400-10,000 cells were backtransplanted into stage 10 chick embryos using several different techniques. The chick embryos were examined at day 11 of incubation for cardiovascular anomalies. Backtransplantation of approximately 1000 cells after 24 hours *in vitro* resulted in the highest percentage of normal cardiovascular development. This system will allow evaluation of the role of single genes in cardiovascular development.

CI 121 THE DEVELOPMENTAL STAGE-SPECIFIC EXPRESSION OF MYOSIN ISOFORMS IN TURKEY MUSCLES, K. Maruyama and N. Kanemaki, USDA, ARS, Avian Physiology Laboratory, Beltsville, MD 20705
The appearance of myosin isoforms in turkey skeletal muscles was studied to characterize the genetic programming for muscle development. Myosin extract was prepared from skeletal muscles of turkeys at various ages from 24-day embryo to adults and the appearance of myosin isoforms was revealed using monoclonal antibodies. Protein slot blotting, in combination with peptide mapping, was used to determine the specificity of monoclonal antibody for turkey myosin isoforms and the enzyme-linked immunosorbent assay was used to determine the developmental stage-specific expression of myosin isoforms. The monoclonal antibody, AB8 was specific for myosin isoform in M. Pectoralis, a fast-twitch muscle, of adult turkeys and the monoclonal antibody 2E9 was specific to myosin isoform expressed during the neonatal stage. The latter antibody was reactive with the neonatal myosin isoform from muscles of a mixed fiber type as well as fast-twitch muscle. Two other monoclonal antibodies, EB165 and AG6, were found to react with myosin isoforms in muscles of turkeys at all ages. The neonatal stage-specific isoform was noted at 4 days after hatch and persisted through 21 days of age and the adult isoform appeared at 2 weeks of age, marking the period of 14 days to 21 days as the transition period for myosin isoform expression.

CI 122 MEIOTIC GENE CONVERSION IN CHICKENS AND ITS IMPLICATIONS FOR THE INTRODUCTION OF FOREIGN DNA INTO THE AVIAN GERM LINE BY HOMOLOGOUS RECOMBINATION. Wayne T. McCormack and Craig B. Thompson, Howard Hughes Medical Institute, Departments of Internal Medicine and Microbiology/Immunology, University of Michigan Medical Center, Ann Arbor, MI 48109.

Specific genes may be targetted for deletion from the mammalian genome by homologous recombination in EC cells. Considerable evidence suggests that the molecular mechanisms by which homologous genetic exchange occurs involve the creation of Holliday junction recombination intermediates, which can be resolved as either a gene conversion or crossover product. In yeast, homologous recombination and gene conversion occur in meiotically active cells and require the activity of a number of distinct proteins. To investigate whether the germ line tissues of avian species are capable of performing similar recombination events, we investigated the immunoglobulin light chain locus from three distinct strains of chicken. Although these strains were derived from the same breeding stock, we have found evidence for the occurrence of at least 10 meiotic gene conversion events and one crossover event during a relatively brief evolutionary time. These data demonstrate that germ line cells derived from chickens undergo active homologous recombination. Our results suggest that homologous recombination-based methods may be an effective means of introducing foreign DNA into chickens.

CI 123 HYPERVARIABLE MARKERS IN THE CHICKEN GENOME, Christine Miller, Michael W. Bruford, Terry Burke,
Department of Zoology, University of Leicester, University Road, Leicester LE1 7RH, U.K.
The distribution and abundance of hypervariable DNA markers in the chicken genome was examined using multilocus DNA fingerprinting. The highly variable loci detected using human minisatellite-derived Jeffreys' probes 33.6 and 33.15 were found to be dispersed in the chicken genome. Segregation analysis in a chicken family containing two parents and 13 offspring showed minisatellite alleles to segregate independently; band transmission followed mendelian expectations. A low level of cosegregation (6.2%) and apperent allelism (13.8%) was found. The minisatellite sequences clearly represent a dispersed, independently segregating set of markers in the chicken genome. Other multilocus probes detect other independent markers in the chicken. 25 polymorphic single locus markers have successfully been isolated from two chicken genomic libraries. In four unrelated individuals these probes detect loci with observed heterozygosity values of 80% to 100%. It is proposed to use locus-specific probes in genome mapping and, possibly, strain identification. The localization of quantitative trait loci and the use of marker-assisted selection seem likely to become possible, provided that the minisatellite loci are sufficiently dispersed and adequate numbers can be isolated.

CI 124 THE DEVELOPMENT OF THE CHICKEN IMMUNE SYSTEM : INVESTIGATIONS ON THE EXPRESSION AND POSSIBLE IMMUNOLOGICAL SIGNIFICANCE OF ALPHA FETOPROTEIN FOR T-CELL DEPENDENT IMMUNE FUNCTIONS, Ulrich Neumann and Larry D.Bacon, Clinic for Poultry, Hannover School of Veterinary Medicine, 3000 Hannover 71, Federal Republic of Germany, and Regional Poultry Research Laboratory, USDA, East Lansing, Michigan 48823, USA

In mammalians, alpha fetoprotein is expressed during fetal life and gravidity under normal conditions. Until recently, this protein was known to possess immunomodulatory properties in-vitro in mammalians only. This has stimulated the question whether alpha fetoprotein plays a role exempting the fetus from the maternal graft-versus-host reaction. The absence of maternal-fetal feedback mechanisms in the chicken, however, would question this hypothesis as relevant at least for the avian system. Quantitatively, chicken alpha fetoprotein (ch-afp) represents approx. 43% of the total serum protein at day 7 of embryonation and thereafter is decreasing gradually and almost absent after hatching. To study the possible immunoregulatory role of ch-afp, in-vitro experiments were performed employing mixed lymphocyte cultures. The presence of physiological quantities of purified ch-afp **significantly** ($p < 0,05$) suppressed allogeneic one-way mixed lymphocyte reactions (MLR) using SPF chicken inbred lines $15I_5$ and line C as donors. Suppression was depending on the presence of ch-afp in the early phase of lymphoblastogenesis in-vitro. Also, sera from chicken embryos of the 12th and 15th day of incubation of four different inbred lines were found to exert MLR suppression. Thus, among other functions, ch-afp may play an immunoregulatory role by maintaining a certain stage of self tolerance during the differentiation of the avian immune system.

CI 125 LIPOSOME-MEDIATED GENE TRANSFER INTO CHICKEN EMBRYOS *IN OVO*
Charles I. Rosenblum and Howard Y. Chen, Department of Growth Biochemsitry and Physiology, Merck, Sharp and Dohme Research Laboratories, Rahway, NJ 07065

We are using liposomes to transfect exogenous genes into early chicken embryos. Fresh-fertile Leghorn embryos were transfected with a solution containing cationic liposome and the plasmid pRSVL which consists of the Rous sarcoma virus long terminal repeat (LTR) and the firefly luciferase gene. The solution was microinjected into the subgerminal cavity beneath the epiblast and treated embryos were incubated in normal fashion for further development. One liposome, TranfectAce™, consistently produced high levels of luciferase, up to 0.15 pg/ug protein, in day 3 embryos and detectable activity in day 8 embryos.

This method is suitable for the examination of promotor activity and effects of foreign gene expression in early embryos. Liposome-mediated gene transfer also provides an alternative approach for producing transgenic avians.

CI 126 DEVELOPMENTAL AND TISSUE-SPECIFIC PROTEIN INTERACTIONS WITH A DNA ELEMENT UPSTREAM OF THE AVIAN APOVLDLII GENE, Aimee K. Ryan and Roger G. Deeley, Cancer Research Laboratories, Queen's University, Kingston, Ontario, Canada, K7L 3K8

Expression of the avian very low density apolipoprotein II (apoVLDLII) gene is estrogen dependent and limited to the liver. Its ability to respond to estrogen stimulation is acquired between days 7 and 9 of embryogenesis. Developmental alterations in DNaseI hypersensitive sites and methylation have been found in the 5'-flanking region of the apoVLDLII gene. The boundaries of protein binding domains have been defined using exonuclease III footprinting. One of these sites is located at -1.96 kb, close to an estrogen dependent DNaseI hypersensitive site. Nuclear protein extracts prepared from embryonic (liver) and adult (liver, kidney, brain, oviduct and blood) tissue samples have been analyzed for binding to this site using gel mobility shift assays. We have shown the following: (1) the level of binding activity is highest in early embryonic livers and decreases during development; (2) in adult tissues, the binding activity is not dependent on the hormonal status of the bird and it is higher in non-liver extracts than in adult liver but still significantly lower than in embryonic extracts; and, (3) the mobilities of the retarded fragments are developmentally-dependent and tissue-specific. Heat denaturation and competition experiments have shown that the protein which binds to this site is not C/EBP, NF-1, HNF-1 or one of the octamer binding proteins. We are currently investigating the hypothesis that binding to this site is involved with suppression of apoVLDLII during embryogenesis. The binding protein has been partially purified and studies are underway to isolate a cDNA clone of this protein. In addition, monoclonal antibodies against this protein are being prepared and will be used to aid in the characterization of this binding protein and its function.

CI 127 REGULATION OF THE CHICKEN APOVLDLII GENE: DETERMINANTS OF HORMONAL AND TISSUE SPECIFICITY. T.J. Schrader, R.A. Burtch Wright and R.G. Deeley. Cancer Research Laboratories, Queen's University, Kingston, Ontario K7L 3N6

ApoVLDLII is a major estrogen-inducible, avian yolk protein that is synthesized exclusively in liver. We have examined the ability of 5' flanking regions extending over 5 kb to determine both the efficiency and tissue specificity with which the gene is expressed, using short term expression assays in primary cultures of various cell types. In order to identify DNA sequences important for hormonal control of apoVLDLII expression, 4.5 kb of 5' flanking region was cloned into pSV2CAT and a series of ExoIII deletion constructs and specific site mutations were generated. The proximal promoter region of the gene contains a canonical estrogen response element (ERE) as well as a second imperfect ERE located only 30 nucleotides upstream. Estrogen responsiveness was almost totally dependent on the single canonical ERE but the level of expression and tissue specificity was largely dictated by upstream sequences that we have correlated with previously mapped protein binding and nuclease hypersensitive sites. Expression from the apoVLDLII promoter can be suppressed in a dominant and ligand dependent fashion by several forms of the retinoic acid receptor (RAR) and RAR-estrogen receptor chimeras. Its activation appears to be specific for the ligand binding domain of the estrogen receptor since conversion of the canonical ERE to a GRE does not confer responsiveness to a GAL4-glucocorticoid receptor (GR) fusion protein, nor to induction by endogenous (GR). However, additional conversion of the second potential ERE to a GRE does render the promoter highly responsive to transactivators with a GR-DNA binding domain.

CI 128 MECHANISMS CONTROLLING THE DEVELOPMENTALLY-REGULATED EXPRESSION OF GENES ENCODING CHICKEN U4 SMALL NUCLEAR RNA, William E. Stumph, Ihab W. Botros, and Jon H. Miyake, Department of Chemistry and Molecular Biology Institute, San Diego State University, San Diego, CA 92182.

In the chicken genome there are two closely-linked genes, U4B and U4X, that code for distinct sequence variants of U4 small nuclear RNA (snRNA). These two genes are differentially regulated during chicken development in that the U4X gene is specifically down-regulated relative to U4B as development proceeds from the embryo to the adult. To examine the molecular basis for this differential expression, we have been studying the interaction of sequence-specific DNA binding proteins with the regulatory sequences of these two genes. First, we have found that the snRNA gene enhancer-binding protein, SBF, binds with a several-fold lower affinity to the U4X enhancer than to the U4B enhancer. Thus, one mechanism that could account for the down-regulation of the U4X gene during development is a model in which SBF is a limiting factor in adult tissues, but not in embryonic tissues. We have also identified a second factor, PPBF, that binds specifically to the proximal regulatory region of the U4X gene, but not to the corresponding regulatory region of the U4B gene. This factor appears to be specifically involved in controlling the expression of the U4X gene and thus provides a second mechanism to differentially regulate transcription of the U4B and U4X genes during chicken development.

CI 129 CHARACTERIZATION OF NEW GENETIC MARKERS FOR POULTRY BREEDING BY MOLECULAR GENOTYPING OF THE MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) OF SELECTED CHICKEN LINES Pierrick Thoraval, Anne-Marie Chaussé, Françoise Coudert and Ginette Dambrine, Station de Pathologie Aviaire et de Parasitologie, INRA, Nouzilly 37380 Monnaie, France.

The chicken MHC (B complex) has been shown to control many biological phenomena, including the resistance or susceptibility to the development of viral induced-tumors. In chicken, the B complex consists of three subregions encoding respectively BF (class I), BL (class II) and BG (class IV) antigens. The availability of molecular probes, specific for these three loci, allowed us to analyse DNA polymorphism between different haplotypes. The B characterized chicken lines used for that purpose have been defined on the basis of hemagglutinating antisera, containing mainly BG antibodies. Restriction fragment lenght polymorphism (RFLP) associated with BG, BF and BLß genes were found for B4, B12, B13, B19 and B21 haplotypes, providing thus new genetic markers for poultry breeding. All the animals that we have analyzed within each serologically defined haplotype share identical RFLP patterns as detected with all the probes used, with the exception of the B19 and B21 haplotypes. On the contrary, in the B19 and B21 chicken lines, we have been able to define several subtypes with BF and BLß probes whereas the animals were identical with the BG probe. The relevance of this finding is under current analysis to determine if these different subtypes are expressed and if they would be implicated in the genetic control of viral-induced tumors.

CI 130 ISOLATION OF CHICKEN cDNAS ENCODING HOMEODOMAIN PROTEINS AND CHARACTERIZATION OF THEIR TEMPORAL AND SPATIAL EXPRESSION DURING EMBRYONIC LIMB DEVELOPMENT, William B. Upholt, Robert A. Kosher, Meyer Barembaum, Kimberly J. Blake, Caroline N.D. Coelho, Siew-Ging Gong, Joann Paiva-Borduas, Barbara J. Rodgers, and Lauro Sumoy, Departments of BioStructure and Function and Anatomy, University of Connecticut Health Center, Farmington, CT 06030

Homeobox-containing genes are thought to be involved in the regulation of pattern formation during vertebrate limb development. Because of its accessibility to microsurgical manipulation, the developing chick limb provides a powerful system for investigating the role of homeobox-containing genes in patterning events. We report the isolation from a chick limb cDNA library of homeobox-containing cDNAs which, on the basis of their nucleotide and deduced amino acid sequences, have been identified as the chicken cognates of mouse Hox-1.4, -2.2, -4.2, -7.1, and -8. We have begun to examine the expression of these genes during chick limb development. G(Gallus)Hox-1.4, -4.2 and -8 are expressed in a graded fashion along the A-P axis of the chick wing bud at the time when positional values along the A-P axis are being specified with high levels of expression occurring in the anterior mesoderm of the limb, and progressively less expression occurring in more posterior regions of the limb mesoderm. This pattern is complementary to that of the proposed morphogen originating from the zone of polarizing activity. GHox-8 undergoes a dramatic decline in expression in mesenchyme coincident with the initiation of limb cartilage differentiation in vitro suggesting that its expression might be incompatible with chondrogenic differentiation. GHox-8 is also expressed in relatively high amounts by the apical ectodermal ridge suggesting its possible involvement in AER function. This work was supported by grants HD22896 and HD22610 to RAK and WBU.

CI 131 TURKEY PROLACTIN AND GROWTH HORMONE: MOLECULAR CLONING, CHARACTERIZATION AND ASSOCIATED RFLPs. D. Zadworny, C. N. Karatzas and U.Kuhnlein, Dept. of Animal Sci., McGill University, Ste-Anne de Bellevue, P.Q., H9X 1C0, CANADA.

Prolactin (PRL) and growth hormone (GH) are structurally related proteins which are believed to have evolved from a common ancestral gene. We have cloned the turkey PRL and GH genes from a pituitary cDNA library. The nucleotide sequences indicate that mature tPRL and tGH consist of 199 and 191 amino acids, with calculated molecular weights of 22,785 Da and 22,248 Da, respectively. Both turkey PRL and GH share about 30%-80% homology at the protein level when compared with other vertebrate members of the gene family. Fourty six amino acids are conserved in tPRL and tGH, including the four cysteines. This supports the theory for divergence of these proteins from a common gene. Turkey PRL and tGH cDNAs were expressed in E.coli using the pGEX-3x vector which carries the tac inducible promoter fused to glutathione S-reductase (GST) and a recognition site for the blood coagulation Factor X_a. The highly expressed soluble fusion proteins ($\sim$50% of the total bacterial proteins) were digested with Factor X_a to cleave the GST carrier and immunoreacted with tPRL and tGH antisera on western blotting. The two cDNA clones were used as probes to search for polymorphisms in turkeys as well as in chickens, where well defined strains were available. No polymorphisms were observed in the PRL gene, but the GH gene was extremely polymorphic in both chickens and turkeys. We have identified at least six MspI polymorphisms with different frequences in egg layers and broiler birds. In Leghorns, GH was polymorphic for two alleles and one appeared to be associated with the onset of laying. Analysis of broilers of a fat and lean line derived from a common genetic base revealed MspI polymorphisms which were only present in the lean line.

Late Abstract

CHARACTERIZATION OF RAV-RELATED ENDOGENOUS VIRAL GENES IN SIX EXPERIMENTAL STRAINS OF CHICKEN. Michèle H. Tixier-Boichard, Lucien Durand. Laboratoire de Génétique Factorielle, Centre de Recherches INRA, 78352 Jouy-en-Josas, Cedex, FRANCE.

RAV-related endogenous viral genes have been already well described in White Leghorn strains. These genes may interfere with susceptibility to leukosis. They may also be used as marker genes to compare populations or lines chosen for their different genetic backgrounds or selection objectives. Here, we describe the RAV-related ev genes of 6 experimental strains, including a White Leghorn, an Egyptian Fayoumi, two Rhode Island Red lines divergently selected for feed efficiency, a Wyandotte and a control broiler population. An average of 70 birds, both sires, dams and their progeny, has been studied for each strain. Genomic DNA was digested with SacI and hybridized with a RAV-2 probe (a gift from L. Crittenden). A set of 28 different restriction fragments was revealed over the 6 strains studied, each of them showing from 7 to 12 fragments. The average number of fragments per individual ranged from 3 to 6 with a maximum of 9. The mean frequency of the SacI fragments ranged from 28% to 68% according to the strain. The average level of heterozygosity could be estimated to lie between 40% and 89%, depending on the strain, with lower values in the more inbred strains. Matching the SacI fragments with the restriction pattern obtained with another enzyme, BamHI, made possible a comparison with ev genes known in White Leghorns. In the Fayoumi strain, for instance, 6 ev genes appeared to be quite new. The use of crossbreeding experiments and of locus-specific probes may help to identify new alleles and loci.