THE MOLECULAR BASIS FOR DIFFERENCES BETWEEN THE SEXES

Organizers: Bruce S. Baker, Barbara J. Meyer and Robin Lovell-Badge

February 12-18, 1995; Tamarron, Colorado

Plenary Sessions *Page*

Poster Sessions

The Molecular Basis for Differences Between the Sexes

Molecular Basis for the Primary Determination of Sex I

B2-001 SRY MUTANTS, PARTNERS, AND RELATIVES

Vincent R. Harley, Department of Genetics, University of Cambridge, Downing Street, Cambridge CB2 3EH, England.

The human testis determining factor, *SRY*, is a Y chromosome-encoded gene which commits the indifferent gonad to the testis determining pathway during mammalian development. *SRY* encodes an HMG box and is therefore most likely to act as a transcription factor. SRY produced *in vitro* is a sequence-specific DNA binding protein; random oligonucleotide selection experiments show that SRY prefers the sequence A/T A A C A A A/T. Mutations in SRY occur in patients with gonadal dysgenesis and the DNA binding activity of SRY encoded by such "XY females" is reduced or abolished, which suggests that this activity is crucial for testis formation. SRY has DNA bending activity and studies of mutant SRY proteins suggest that the DNA binding and DNA bending activities can be decoupled. The structural implications of SRY-DNA interaction will be considered in the light of the nmr structure of the HMG box of HMGI.

SRY is nuclear-localised when expressed in mammalian cells; recently we discovered a protein which specifically interacts with SRY at sites thought to be responsible for nuclear entry.

SOX9, an *SRY*-related HMG box gene, has been cloned and mapped to chromosome 17q24, which coincides with the locus associated with campomelic dysplasia and, intriguingly, with autosomal sex reversal. Recombinant SOX9 was produced in bacteria and its biochemical activities will be discussed.

B2-002 HUMAN SRY AND SEX REVERSAL, Ken McElreavey, Sandrine Barbaux, Eric Vilain and Marc Fellous, Immunogénétique Humaine, Institut Pasteur, 25 Rue du Dr Roux, 75724 Paris Cedex 15, France.

Pathologies of human sex reversal can be classified into 46,XX males, 46,XX and 46,XY true hermaphrodites and 46,XY females with gonadal dysgenesis.The latter group can be further subdivided into either complete or partial 46,XY gonadal dysgenesis. The majority of 46,XX males carry the *SRY* gene on one of their X chromosomes as a result of an unequal crossing-over event between the X and Y chromosomes during male meiosis. In contrast a minority of 46,XX true hermaphrodites carry the *SRY* gene. The presence of *SRY* in an XX individual results in a more masculinised phenotype. Familial cases of *SRY*-negative 46,XX sex reversal also occur. The pattern of transmission of the sex reversed phenotype in these pedigrees is compatible with a recessive mode of inheritance. These observations lead us to propose a simple model of mammalian sex determination; SRY may function as a repressor of a negative regulator of male development.This model will be discussed taking into account recent observations of duplications of critical region of the X chromosome (Xp21.3) in certain XY females.

Nonsense and missense mutations have been described in *SRY* coding sequences associated with 46,XY complete gonadal dysgenesis. However other rearrangements in the testis determining region of the Y chromosome outside the *SRY* gene can also result in XY sex reversal. Microdeletions 5' and 3' to *SRY* are associated with 46,XY complete and 46,XY partial gonadal dysgenesis respectively. These deletions may define regulatory elements. Finally a case of a 46,XY female will be described who carries a microduplication of the *SRY* gene in addition to the wild-type *SRY* gene. The functional implications of these findings will be discussed.

The Functioning and Organization of the Regulatory Hierarchies that Govern Sex in Somatic Cells I

B2-003 AUTOSOMAL GENES INVOLVED IN MAMMALIAN SEX DETERMINATION, Eva M. Eicher[1], Linda L. Washburn[1], Xiaoling Xu[2], Robert D. Dredge[2], Nicholas J. Schork[1,3], Kenn H. Albrecht[1], L. Christine Turtzo[1], Peter C. Hoppe[1], M. Jodeanne Pringle[2], Elaine P. Shown[1], Barbara K. Lee[1], and David C. Page[2], [1]The Jackson Laboratory, Bar Harbor, ME 04609, [2]Howard Hughes Laboratories and Whitehead Institute for Biomedical Research, Cambridge, [3]Case Western University, Cleveland, OH.

Transfer of the *Mus domesticus poschiavinus* Y chromosome (Y^{POS}) to the C57BL/6J inbred strain background causes all XY mice to develop ovarian tissues. Half of C57BL/6J-Y^{POS} individuals develop exclusively ovarian tissue (XY females) and half develop testicular and ovarian tissue (XY hermaphrodites). Transfer of the Y^{POS} chromosome to other inbred strain backgrounds, such as DBA/2J or BALB/cByJ, results in normal testicular development in all XY individuals. Our investigation of this sex reversal condition suggested that it is inherited as a complex trait, having a Y-linked component and autosomal components. We used transgenic technology to determine that the *Sry* gene carried by the Y^{POS} chromosome is responsible for the Y-linked component. We conducted a genetic mapping experiment involving the DBA/2J and C57BL/6J-Y^{POS} strains to identify the autosomal genes. Two genes were mapped, one to Chromosome 2 and one to Chromosome 4. A third gene may be located on Chromosome 5.

The Molecular Basis for Differences Between the Sexes

The Functioning and Organization of the Regulatory Hierarchies that Govern Sex in Somatic Cells II

B2-004 FUNCTIONS AND REGULATION OF THE *tra-1* GENE OF *C. elegans*. Jonathan Hodgkin, David Zarkower, Rachel Aronoff, and Mario de Bono, MRC Laboratory of Molecular Biology, Cambridge CB2 2QH, England.

The *tra-1* gene occupies a terminal position in the cascade of regulatory genes controlling somatic sexual phenotype in the nematode C. *elegans*, and it also has significant functions in the germ line. The major function of the gene is to dictate female somatic development: high activity of *tra-1* is necessary and sufficient for female development in all tissues, and low or zero activity is necessary and sufficient for male development. The gene has two transcripts, a 5kb transcript encoding TRA-1A, a protein containing five tandem zinc fingers, and a shorter message encoding TRA-1B, which has only the first two zinc fingers. Several lines of evidence suggest that most or all of *tra-1* activity is mediated by TRA-1A. DNA binding preferences of TRA-1A *in vitro* have been determined, with results very consistent with structural work (by Pavletich and Pabo) on the human GLI protein, which contains five zinc fingers highly similar in sequence to those of TRA-1A. The simplest hypothesis for *tra-1* action is that TRA-1A acts as a transcriptional regulator, controlling a variety of target genes to direct female development (when active) or permit male development (when inactive). Target genes are being sought in two ways: first by examining genes with specific roles in male and female development, such as *mab* (male abnormal) or *egl* (egg-laying defective) genes. Examples are *mab-3*, which affects male tail development and yolk protein synthesis; and *egl-1*, which affects sex-specific cell deaths. Second, the DNA-binding consensus of TRA-1A can be used to explore the large (>5 Mb) sequence database of *C. elegans*. One notable candidate identified in this way is the Hox cluster gene *egl-5*, which has two upstream consensus sites, and appears (from data obtained by Wang, Kenyon et al.) to be transcribed at higher levels in males than in hermaphrodites. With regard to regulation, genetic data imply that the activity of *tra-1* must be regulated so that it is high in XX (hermaphrodite) and low in XO (male). The major regulation appears to be post-transcriptional. Most gain-of-function mutations of *tra-1* (which act as dominant constitutively feminizing mutations) have missense changes in a short (16aa) domain, close to the amino-terminus of TRA-1A. An additional minor regulatory site may be located close to the carboxy-terminus. These and other results suggest that TRA-1A activity is negatively regulated in XO animals by protein-protein interactions with other nuclear proteins, such as products of one or more of the *fem* genes, which are predicted to act as negative regulators of *tra-1* activity.

B2-005 FUNCTION AND EXPRESSION OF THE *C. elegans* MASCULINIZING GENE *her-1*, Marc D. Perry[a,b], Barbara Robertson[a], Carol Trent[c], Pepper Schedin[a] and William B. Wood[a]. [a]Dept. of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, CO 80309, [b]Dept. of Molecular and Medical Genetics, University of Toronto, Toronto, Ontario, Canada M5S 1A8, [c]Dept. of Biology, Western Washington University, Bellingham, WA 98225.

During *C. elegans* embryogenesis the *her-1* gene's activity is modulated by the X:A ratio. When *her-1* activity is high the activity of *tra-1*, the terminal switch gene in this pathway, is repressed, permitting male development. Conversely, if *her-1* activity is repressed by the X:A ratio, then *tra-1* functions to direct female somatic development. *her-1* exerts this masculinizing effect non-cell-autonomously, and our molecular analysis revealed that one of the gene's two male-specific transcripts encodes a small, novel, Cys-rich secreted protein. Now we have identified the DNA sequence changes in each of the 32 extant *her-1* alleles. Intriguingly, six alleles map to the promoter region, including two identical neomorphic mutations which allow expression of *her-1* in hermaphrodites, and seem to define a site where *her-1* is negatively regulated in XX animals. Three of the four remaining promoter mutations are single base changes that cause temperature-sensitive loss of *her-1* function. Initial experiments to determine the *her-1* TSP were executed using *her-1(e1561ts)*, the strongest of these *ts* alleles, and were interpreted as supporting the idea that the HER-1 protein is required to specify male sexual fates during embryogenesis, but is dispensable during larval growth. Subsequent experiments employing temperature shifts in adulthood revealed that *her-1* has a maintenance function in sex determination. *her-1(e1561ts)* adult XO males shifted from 16C to 25C began to synthesize hermaphrodite-specific yolk proteins in their intestinal cells and oocytes in their germ-line. To ask how late *her-1* can act to masculinize cell fates in XX hermaphrodites we are using a heat-shock-*her-1* fusion gene in transgenic worms. We are complementing this analysis both by repeating the TSP experiments using a missense allele, *her-1(e2296ts)*, and by characterizing anti-HER-1 antisera.

B2-006 THE HIERARCHIES CONTROLLING DIFFERENT ASPECTS OF SOMATIC SEX IN DROSOPHILA, Lisa C. Ryner, Volker Heinrichs, Yann-Pyng Toung, Hao Li, Carrie Garrett-Engele and Bruce S. Baker, Department of Biological Sciences, Stanford University, Stanford.

Somatic sexual differentiation in Drosophila melanogaster is controlled by a hierarchy of regulatory genes. At the core of the hierarchy are the interactions of the *Sex-lethal (Sxl), transformer (tra), transformer-2 (tra-2)* and *doublesex (dsx)* genes. Molecular analysis has revealed that these genes participate in a cascade of alternative RNA splicing decisions that results in the production of sex-specific *dsx* products. We have taken several approaches to study the mechanisms by which this regulatory hierarchy functions. Using a detailed molecular analysis we have determined that *tra* and *tra-2* direct splicing of the *dsx* pre-mRNA by activating use of the 3' splice site of the female-specific exon through their interaction with a 13nt repeated element found within the female-specific exon. To look for other factors that may interact with *tra* and *tra-2* we have used a genetic screen for mutations that enhance an interaction between *tra* and *tra-2*. One candidate from this screen has been cloned and has a striking similarity to a yeast protein involved in chromosome segregation and also contains homology to a hnRNP K type RNA binding domain. Studies of the role for this protein in the control of *dsx* expression are underway. In addition, we have examined the role of a general splicing factor, *rbp1*, in *dsx* pre-mRNA splicing. *rbp1* is a Drosophila homologue of a member of a conserved family of splicing factors (SR proteins) that have been previously implicated in the control of alternative RNA processing decisions in mammals. We have found that *rbp1* has several binding sites in the *dsx* pre-mRNA and can influence the splicing efficiency of the female-specific site. Two other genes that function in the hierarchy are *intersex (ix)* and *hermaphrodite (her)*. *ix* is required in females in addition to *dsx* for proper female development. *her* is a pleiotropic gene that functions both zygotically and maternally for proper sexual differentiation in both males and females. Both of these genes are in the process of being cloned. Finally, we have been involved in the identification of a new branch to the hierarchy. Up to a few years ago it was thought that all aspects of somatic sexual development were controlled through *dsx*, as it lies at the bottom of the regulatory hierarchy. However, this notion has changed due to the discovery made by B. Taylor (1992), who found that the development of a male-specific muscle was not controlled by *dsx* but was controlled by genes upstream in the hierarchy. This result suggested that there was a previously unrecognized branch to the regulatory hierarchy just downstream of *tra* and *tra-2*. We reasoned that the gene at the top of this new branch should contain the same *cis*-acting regulatory sequences found in *dsx* through which *tra* and *tra-2* interact, namely the 13nt repeated element. Using these sequences as a probe we have identified the *fruitless (fru)* gene as a strong candidate for a gene at the top of this new branch of the hierarchy. The *fru* gene was previously shown to be involved in the control of sexual behavior in males as well as the development of the male-specific muscle. Interestingly, both are processes that involve sex-specific aspects of the nervous system. Hence, the *fru* branch to the hierarchy may function to control sex-specific aspects of nervous system development and function. We are presently involved in the molecular characterization of the *fru* gene.

The Control of Somatic Sexual Differentiation, Including Behavior I

B2-007 MüLLERIAN-INHIBITING SUBSTANCE FUNCTION DURING MAMMALIAN SEXUAL DEVELOPMENT

Richard R. Behringer[1], Yuji Mishina[1], Milton J. Finegold[2], Nathalie di Clemente[3], Nathalie Josso[3], and Richard L. Cate[4], [1]Department of Molecular Genetics, The University of Texas, M. D. Anderson Cancer Center, Houston, Texas, [2]Department of Pathology, Baylor College of Medicine, Houston, Texas, [3]Unite de Recherches sur l'Endocrinologie du Développement, Ecole Normale Superieure, Montrouge, France, [4]Biogen, Inc., 14 Cambridge Center, Cambridge, Massachusetts.

To investigate the role of Müllerian-inhibiting substance (MIS), also known as anti-Müllerian hormone (AMH), in mammalian sexual development, we generated MIS-deficient mice. Although MIS-deficient males had testes that were fully descended and produced functional sperm, they also developed female reproductive organs (a uterus, uncoiled oviducts, and vagina), which interfered with sperm transfer into females, rendering most infertile. Their testes had Leydig cell hyperplasia and in one instance neoplasia. The infertility and male pseudohermaphroditism phenotype was rescued by the chronic expression of a human MIS transgene. The actions of the two primary hormones of male sexual differentation were genetically eliminated using the *testicular feminization* (*Tfm*) mutation in combination with the MIS mutant allele. XY *Tfm*/MIS-double mutants developed as females, with a uterus, coiled oviducts, and no male reproductive organs except undescended dysfunctional testes. These results suggest that eliminating the presumptive female reproductive tract in male fetuses facilitates fertility and that in testes MIS is a negative regulator of Leydig cell proliferation. In addition, eliminating the presumptive male reproductive tract is necessary for proper oviductal morphogenesis during female mouse development. To investigate how MIS signals are mediated, we have isolated the mouse MIS/AMH receptor gene to create receptor-deficient mice. These mice will be a useful genetic resource for understanding the ligand/receptor relationships for MIS/AMH that are required for normal sexual development.

The Control of Somatic Sexual Differentiation, Including Behavior II

B2-008 BEYOND TESTIS DETERMINATION IN THE MOUSE: OTHER Y CHROMOSOME FUNCTIONS AND CANDIDATE GENES,

Shantha K. Mahadevaiah[1], Äine Rattigan[1], Teresa Odorisio[1], Steve H. Laval[2], Bruce M. Cattanach[2], Alan R. Thornhill[3], and Paul S. Burgoyne[1], [1]MRC National Institute for Medical Research, London, UK, [2]MRC Radiobiology Unit, Didcot, UK, and [3]Institute of Child Health, London, UK.

The cloning of the Y-chromosomal testis determinant *Sry* was the culmination of a search which involved an interplay between deletion mapping and positional cloning. In the course of this search other Y functions were defined, particularly with respect to spermatogenesis, and a number of Y genes were identified at the molecular level. In the present review the following Y chromosome functions will be discussed: 1) An accelerating effect of the Y on early development. Evidence will be presented that this effect is not due to *Sry*. 2) A Y effect on early spermatogonial proliferation. The most promising candidate gene is *Ube1y* which encodes a ubiquitin activating enzyme known to be important for cell cycle progression. However, the zinc finger genes *Zfy-1 & 2* are also potential candidates. Recently a multiple copy gene (*YRRM*) was identified on the human Y encoding a family of RNA binding proteins (a candidate for the human spermatogenesis factor *AZF*), and probing mouse genomic DNA with a human cDNA probe suggested that this gene is present on the mouse Y. This mouse *Yrrm* was therefore also proposed as a candidate for the spermatogonial proliferation factor. However, we have established that the *Yrrm* sequences on the mouse Y map to the wrong deletion interval. Nevertheless, *Yrrm*-related transcripts are present in testis suggesting a possible role in spermatogenesis. 3) The Y functions as a pairing partner for the X during meiosis, pairing being mediated by the X-Y homologous 'pseudoautosomal region'. This X-Y pairing has been veiwed as a mechanism for ensuring the segregation of the X and Y at the first meiotic metaphase. We have shown that X-Y pairing is also necessary for spermatocyte survival - spermatocytes in which either the X or Y remain unpaired, are eliminated by apoptosis. 4) A Y chromosome effect on sperm head development. Partial deletions of the Y long arm produce an increase in sperm head abnormalities and a distorted sex-ratio, while total absence of the Y specific region of the long arm results in 100% sperm abnormality and sterility. A family of related sequences are detected on the mouse Y long arm by the probe Y353/B, and there are related testis-specific transcripts. We have shown that these transcripts are transcribed from multiple genomic copies and are expressed exclusively in round spermatids. A number of Y353/B-related cDNAs have been cloned and sequenced, and there is a conserved open reading frame. However, no protein product has yet been identified. A model seeking to explain how partial deletions (removing two thirds of Y353/B-related sequences) affect the incidence of sperm head abnormality and distort the sex ratio in favour of females will be discussed.

B2-009 THE FUNCTION OF THE DSX PROTEINS IN THE CONTROL OF SOMATIC SEXUAL DIFFERENTIATION IN DROSOPHILA,

Kenneth C. Burtis[1], Scott E. Erdman[2], HuiJu Chen[1], Charles Whitfield[3], Brian Thornton[1], and Victoria A. Jursnich[1], [1]University of California, Davis, CA 95616, [2]Yale University, New Haven, and [3]Stanford University, Stanford.

Many (but not all) aspects of the somatic sexual differentiation of Drosophila are under the control of the *doublesex* (*dsx*) gene, which lies at the bottom of the hierarchy of regulatory genes controlling sex-specific aspects of Drosophila development. The *dsx* gene expresses alternatively spliced sex-specific transcripts that encode polypeptides sharing a common amino-terminal domain but sex-specific carboxy-termini. These polypeptides possess a zinc-finger related DNA binding domain in the common region, which is essential for biological function of the proteins in both sexes. We have determined the consensus binding site for this domain, which consists of a seven base pair palindromic sequence. We have determined that two molecules of dsx protein bind to this site, consistent with its palindromic nature. Protein crosslinking experiments have indicated that under certain conditions the dsx proteins are dimerized *in vivo*, and we have demonstrated that in transgenic flies expressing both the male-specific and female-specific dsx polypeptides heterodimers can form. We are currently mapping the dimerization domain(s) using the yeast two-hybrid system. We are also using a yeast two-plasmid system developed by J. Lopez of Carnegie-Mellon University to identify regulatory sequences from the Drosophila genome that are potential target sites for the dsx proteins. This method has been used successfully to identify target genes of the *Ultrabithorax* homeobox protein, and involves identifying small genomic fragments of Drosophila DNA that are able to mediate DSX-dependent regulation of an adjacent yeast promoter. The frequency with which such fragments have been found in our screen suggests that the approach is identifying a unique class of DNA sequences, and we have begun the analysis of these sequences to determine if they are relevant to sexual differentiation in Drosophila.

The Control of Somatic Sexual Differentiation, Including Behavior III

B2-010 CONTROL OF MALE TAIL DIFFERENTIATION IN *CAENORHABDITIS ELEGANS*, Scott W. Emmons, King L. Chow, Yinhua Zhang, and Connie Zhao, Department of Molecular Genetics, Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, NY 10461.

Sexual dimorphism presumably arises because a number of genes responsible for aspects of morphology, physiology and behavior are regulated by the sex determination pathway. In *C. elegans*, the last gene in the sex determination pathway is the presumptive transcription factor *tra-1*. Hence, regulation of downstream genes in *C. elegans* is likely to be at the transcriptional level. As yet, *tra-1* target genes have not been identified, and the number of such genes is unknown. Studies of genes necessary for differentiation of sex-specific structures will allow an analysis of whether and how their activities are controlled by the sex determination pathway. We are studying develoment of the genital specializations of the male tail as a model for understanding the genetics and evolution of morphology. Rays are peripheral sense organs present in the male tail necessary for the male to sense contact with the hermaphrodite mating partner. Genetic screens for abnormal males have resulted in the identification of several genes necessary for the generation and correct morphogenesis of the rays. None of these genes functions uniquely in males. The *lin-32* gene, which encodes a bHLH transcription factor of the *achaete/scute* family, is both necessary and sufficient for expression of the ray neuroblast fate, and is necessary as well for several neuroblast fates found in both males and hermaphrodites. Ectopic expression of *lin-32* results in expression of the ray fate in anterior body regions in both males and hermaphrodites. This indicates that *lin-32* may be a key target of both spatial and sexual regulation, as a result of which rays are normally found in only one sex and body region. Two genes of the *C. elegans* HOM-C/Hox gene complex, as well as genes in a putative TGFβ signalling pathway, are necessary for morphogenesis of the rays. Action of these genes results in the assignment of a unique identity for each ray that is necessary to prevent fusion of rays to each other during morphogenesis. For several rays, the ratio of activity of the HOM-C/Hox genes *mab-5* and *egl-5* is important in determining ray identity. Several newly identified genes, including *mab-18*, a homolog of vertebrate *Pax-6*, and *mab-21*, a gene encoding a novel protein, are genetic modifiers of the effects of *mab-5* and *egl-5*. The *mab-18* gene undergoes alternative splicing to generate a variety of forms with functions in several body regions in both sexes. These genetic results are beginning to reveal the elements of the developmental pathway governing generation and differentiation of the rays. Further studies, including molecular analysis of transcriptional regulation, should reveal the points at which the sex determination pathway exerts its control.

B2-011 REGULATION OF DIFFERENTIATION OF SEX-SPECIFIC MUSCLES IN DROSOPHILA BY THE GENES *fruitless* AND *doublesex*, Barbara J. Taylor, Laura M. Knittel, and Thomas S. Merritt. Department of Zoology, Oregon State University, Corvallis OR 97331-2914.

In *Drosophila melanogaster*, two output pathways have been discovered leading from the gene cascade controlling sexual differentiation of somatic tissues[1]. At the head of one pathway, the gene *doublesex* (*dsx*) is involved in the differentiation of many sexually dimorphic tissues, such as the external genitalia. At the head of the second pathway, another gene, *fruitless*, has been proposed to control the differentiation of sex-specific muscles and male courtship behaviors. We are using both genetic and molecular studies to identify the role of these two genes in the developmental of sexually dimorphic muscles and neurons in the central nervous system, tissues that have received relatively little attention. Muscles associated with the male and female genitalia and a large abdominal muscle in males constitute the known set of sex-specific muscles in *Drosophila*. From our studies, it appears that *dsx* plays a role in the differentiation of genital muscles while *fru* regulates the sex-specific development of a male-specific abdominal muscle, the Muscle of Lawrence (MOL). Using mutations of *dsx* that allow the simultaneous appearance of both male and female genitalia, we have determined that only a subset of the normal complement of male- and female-specific genital muscles differentiate. To test whether these muscles require innervation or the presence of specific cuticular attachment points, we are denervating the nerves to the genital muscles before the onset of adult development and using mutations to manipulate the development of cuticular insertion sites. In the case of the other sex-specific muscle, MOLs, which require innervation by male-specific motorneurons, we have shown that MOLs have more muscle nuclei than neighboring muscles or MOL-homologues in females. In contrast, in *fru* mutant males, only small MOL-position fibers form, which have substantially fewer nuclei compared to MOL fibers in wildtype males. The number and distribution of myoblasts in larval *fru* mutants are the same as in wildtype animals suggesting that *fru* mutant males are defective in later events during myogenesis. We propose that a primary role of the fru^+ product in MOL development is in the male-specific recruitment of myoblasts into the MOL myotubes occurring at the expense of neighboring muscle fibers. Ablation of muscle precursors in wildtype males by hydroxyurea was unable to reproduce additional deficits seen in the MOL-position fibers of severe *fru* mutant suggesting that fru^+ functions in other aspects of MOL development. We are testing by transplantation experiments whether it is the myoblasts or in the innervating motorneurons where the *fru* effect is manifest.

1. Taylor, B. J., Villella, A., Ryner, L. C., Baker, B. S. and Hall, J. C. (1994) Developmental Genetics 15:275-296.

Sex Determination in the Germline and Its Relationship to Somatic Sex Determination I

B2-012 GERM CELL SEX DETERMINATION IN MAMMALS, Anne McLaren, Wellcome/CRC Institute, University of Cambridge, UK.

In the mouse, primordial germ cells cease dividing a few days after they have entered the genital ridge. In female embryos, the germ cells then enter prophase of the first meiotic division. In male embryos, they arrest in the G1 stage of the cell cycle and do not recommence mitotic proliferation until after birth. Entry into meiosis is not related to reactivation of the silent X chromosome in XX germ cells, which also occurs shortly after they reach the genital ridge, nor is it related to the chromosome constitution of the germ cell itself. All germ cells appear to enter meiosis at this stage of development unless they are located in or very close to a male genital ridge, suggesting the action of an inhibitory effect emanating from the developing testis, rather than any requirement for a meiosis-inducing substance in female embryos. Once a germ cell has entered meiotic prophase, at this early stage, it is committed to the pathway of oogenesis; any surviving germ cells in mitotic arrest will undergo spermatogenesis. Thus the phenotype sex of germ cells in mammals is not cell-autonomous, but appears to be controlled at least in part by the tissue environment.

Sex Determination in the Germline and Its Relationship to Somatic Sex Determination II

B2-013 TRANSLATIONAL CONTROL AND SEX- DETERMINATION IN C. elegans. *Elizabeth B. Goodwin and Judith Kimble, *Dept of Cell & Mol. Biology, Northwestern U. Med. School, Chicago, IL 60611; HHMI & U. Wisconsin, Madison, WI 53706.

In *C. elegans*, animals with two X chromosomes (XX) are self fertilizing hermaphrodites, while animals with one X chromosome (XO) are male. The *tra-2* gene is required for female development; loss of *tra-2* activity results in XX animals developing as males. In the hermaphrodite germline, *tra-2* is repressed at the translational level to achieve spermatogenesis. Two 28 nt direct repeat elements (DREs), located in the *tra-2* 3' untranslated region (3'UTR), are necessary for translational control. XX gain-of-function *tra-2* mutants have disrupted DREs and develop as females; they make no sperm. In an attempt to identify translational regulators of *tra-2*, we have focused on the *laf-1* gene. Five *laf-1* mutations have been independently isolated. All five cause semi-dominant feminization of the XX germline, recessive lethality, and appear to reduce *laf-1* gene function. Three lines of evidence suggest that *laf-1* may control *tra-2* translation. First, *laf-1* mutations disrupt regulation of a reporter transgene via the *tra-2* 3'UTR. (The transgene contains the *lac-Z* gene fused to the wild-type *tra-2* 3' UTR, and is under control of the heat shock promoter) In a wild-type background, little or no β-gal activity is detected, whereas in a *laf-1/+* mutant background, β-gal is readily seen. Second, double mutant experiments indicate that *laf-1* acts upsteam of *tra-2*, a predicted position of a repressor of *tra-2*. Third, the germline feminization, typical of *laf-1* mutants, is the predicted phenotypic effect of losing the translational inhibition of *tra-2*. In previous studies, *tra-2* and *tra-3* have been inseparable in the genetic hierarchy. The *tra-3* gene, like *tra-2*, promotes female development, and loss of *tra-3* activity leads to male development. However, the *laf-1/+; tra-3(lf)* double mutant is feminized in both germline and soma. The simplest interpretation is that *tra-3* may act upstream of *laf-1*. We suggest that *tra-3* promotes the female fate by inactivating *laf-1* and thereby removing *tra-2* from translational repression.

B2-014 SEX, DEATH AND TUMORS IN THE DROSOPHILA GERM LINE: A HIERARCHY OF SEX DETERMINATION (AND DOSAGE COMPENSATION?), Brian Oliver, Department of Zoology and Animal Biology, University of Geneva, 154 bis route de Malagnou, CH-1224 Chêne-Bougeries, Switzerland.

The analysis of germ-line sex determination in Drosophila has lagged the somatic sex determination studies largely because it was not clear what to expect of a germline sex transformation phenotype. Genetic and molecular studies suggest that at least some members of the ovarian tumor class of mutants are defective in germ-line sex determination and might result in germline tumors due to an inappropriate gonadal environment (e.g. spermatocytes in an ovary). One gene that frequently mutates to yield an ovarian tumor phenotype is *Sex-lethal*, a key regulator of somatic sex determination. As in the soma, the *Sex-lethal* gene is regulated by sex-specific pre-mRNA splicing. Chromosomal females with ovarian tumors caused by *ovo*, *ovarian tumor*, or *sans fille* show male-specific *Sex-lethal* pre-mRNA splicing suggesting that the wild type function of these genes in the sex determination hierarchy is to facilitate female-specific *Sex-lethal* expression in ovaries. Genetic studies have also shown that female germ-line sex determination requires a female soma. To determine if this signaling requires all or some of the somatic sex determination pathway the pre-mRNA splicing of *Sex-lethal* was examined in chromosomal females somatically transformed to male by mutations in the *transformer*, *transformer-2* or *doublesex* genes. Both male-specific and female-specific *Sex-lethal* pre-mRNA splicing was detected suggesting that these genes are required for full female-specific *Sex-lethal* expression. The incomplete sex transformation phenotypes observed in the case of the somatic sex determination mutations and the ovarian tumor mutations may mean that the two pathways are reinforcing.

Function of the somatic sex determination genes and some of the germ line sex determination genes are required for good female germ line viability. It is not known what the viability function is, but a hypothesis is that these genes regulate germ line dosage compensation. As a gene required for dosage compensation should be required in flies based on the sexual karyotype, not the sexual phenotype, the requirement for one germline sex determination gene, *ovo*, has been examined in flies where these features do not match. The *ovo* gene encodes a highly conserved zinc finger protein of the C_2H_2 type and is required for the viability of Drosophila germ cells with an XX karyotype. It is not required for the viability of germ cells with an XY karyotype irrespective of the sexual identity exhibited by the soma. It is not required in XO males. Genetic data link *ovo* to the *maleless* gene product, which is involved in dosage compensation in the soma and is required in XY male germ cells. These data indicate that *ovo* is required specifically in XX germ cells. Dosage compensation genes would certainly be expected to show either XX or X0 functional specificity. The *ovo* gene also responds to the number of X-chromosomes. Several *ovo:lacZ* reporter genes are expressed at high levels in females and at low levels in males. The analysis of flies with mismatched sexual karyotypes and sexual phenotypes suggests that these level differences are due to the number of X-chromosomes. We have not identified the X-linked genes or sites used to count X-chromosomes, but we are investigating *ovo* itself because dominant negative alleles of *ovo* cause down-regulation of *ovo:lacZ* reporter activity, and *ovarian tumor* because XX females with no *ovarian tumor* gene show patchy *ovo:lacZ* activity and because *ovarian tumor* has as a dose dependent effect on dominant negative *ovo* alleles.

B2-015 EARLY EVENTS IN GERMLINE SEX DETERMINATION IN DROSOPHILA, Monica Steinmann-Zwicky, Astrid Heller, Eva Niederer, Marylène Poirié and Susanne Staab, Zoological Institute, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland.

In *Drosophila* the sex of germ cells is determined by cell-autonomous and inductive signals. When XX germ cells are transplanted into male hosts, they become spermatogenic, which shows that they respond to induction. XY germ cells, however, that develop in a female host are also spermatogenic: thus, a cell autonomous signal makes XY germ cells male (1). The genes that determine the sex of somatic cells in *Drosophila* control the production of inductive signals. An XY host that is transformed into a female due to ectopic expression of *tra* can have ovaries with oogenic XX donor germ cells (2). Most of the genes that determine the sex of somatic cells in *Drosophila*, however, are dispensable within germ cells (3, 4, 5, 6). The product of *Sxl* is required for germ cells to become oogenic (7, 1). *Sxl* is a target of an inductive sex-determining signal (1, 8). Since, however, larvae lacking the germline-specific function of *Sxl* possess gonads that are indistinguishable from female gonads, *Sxl* might be required only late for oogenesis (9).

Germ cells already display sexual dimorphism in embryos. When the gonads are formed, prospective testes integrate significantly more germ cells than prospective ovaries. To find genes that are involved in early germline sex-determination, we have screened enhancer-trap lines for specific expression in the early gonads. One enhancer-trap line is specifically expressed in stem cells of the male germline. This marker is already expressed in embryonic germ cells. It therefore reflects the expression of one of the earliest sex-specifically expressed germline genes. XX germ cells express the male-specific marker in masculinized XX flies. The expression of this marker is therefore controlled by induction in XX germ cells. XY germ cells express the marker in feminized XY flies: these cells must express the marker autonomously. Thus, autonomous and inductive signals determine the sex of germ cells already in embryos. We are currently testing the expression of this male-specific marker in animals carrying various mutations. Our results suggest that the sex of germ cells is not irreversibly determined until late and that there are early and late events in germline sex determination.

Ref: 1, Steinmann-Zwicky et al., 1989, Cell **57**: 157; 2, Steinmann-Zwicky, 1994, Dev. **120**: 707; 3, Marsh and Wieschaus, 1978, Nature **272**: 249; 4, Schüpbach, 1982, Dev. Biol. **89**: 117; 5, Cronmiller and Cline, 1987, Cell **48**: 479; 6, Steinmann-Zwicky, 1993, Dev. **117**: 763; 7, Schüpbach, 1985, Genetics **109**: 529; 8, Oliver et al., 1993, Dev. **119**: 897; 9, Steinmann-Zwicky, 1994, Dev. Genet. **15**: 265.

Sex Determination in the Germline and Its Relationship to Somatic Sex Determination III

B2-016 THE *gld-1* GENE AND GERMLINE SEX DETERMINATION IN *C. elegans*. Allan Jones, Ross Francis, Robert Clifford and Tim Schedl, Department of Genetics, Washington University School of Medicine, St. Louis MO.

The *gld-1* gene of *C. elegans* controls multiple aspects of germ cell development. A major function of *gld-1*(+) is to direct oocyte development. In *gld-1(null)* hermaphrodites, oogenesis is abolished and a germline tumor forms where oocyte development would normally occur. By contrast, males are unaffected by *gld-1(null)* mutations. The hermaphrodite germline tumor derives from germ cells that enter the meiotic pathway normally, but then exit pachytene and return to the mitotic cycle. Genetic epistasis analysis demonstrates that tumor formation is dependent on the sexual fate of the germ line, but not on chromosomal sex or phenotypic sex of the soma. When the germline sex determination cascade is set in the female mode (terminal *fem/fog* genes inactive) *gld-1(null)* germ cells exit meiotic prophase and form a tumor, but when the pathway is set in the male mode they develop into sperm. Thus, the *gld-1*(+) function necessary for oogenesis acts downstream of, and in response to, the germline sex determination pathway. In this capacity, *gld-1*(+) could function either to specify the oocyte fate or to execute an early essential step in oocyte differentiation.

gld-1(+) has an additional, non-essential function in germline sex determination, the promotion of hermaphrodite spermatogenesis. This *gld-1* activity was inferred from a haplo-insufficient phenotype and from the properties of gain-of-function (*gf*) mutations which cause transformations in germ cell sexual fate. Genetic epistasis experiments using the *gld-1 gf* mutations are consistent with the proposal that *gld-1*(+) promotes spermatogenesis in the hermaphrodite by assisting the activity of the terminal *fem/fog* genes.

Molecular analysis indicates that *gld-1* encodes a germline-specific product and confirms that the tumorous germline phenotype results from the elimination of gene function. The predicted protein contains a 150 aa region with significant similarity to the mammalian protein GAP-associated p62 (GAPap62). However, the *gld-1* protein sequence is not similar in the regions of GAPap62 involved in binding the SH2 and SH3 domains of GAP and other proteins. Within the larger region of similarity is a more loosely conserved motif (~30 aa) termed the KH domain, which has been found in a number of proteins that exhibit RNA and/or DNA binding. The importance of the conserved region and the KH domain in *gld-1* function is indicated by missense mutations which eliminate gene function and/or have *gf* interfering effects on promotion of hermaphrodite spermatogenesis. Antibody staining reveals that GLD-1 is a cytoplasmic protein. If *gld-1*(+) binds nucleic acids as suggested by the conservation with other nucleic acid binding proteins, it may affect RNA metabolism in the germline, possibly by controlling the translation or stability of target mRNAs. The *gld-1* gene product is found in germ cells that will give rise to both sperm and oocytes, but expression is spatially restricted within the germ line.

Control of the Expression of Sex Chromosomes (Dosage Compensation) I

B2-017 FUNCTION OF THE MOUSE *XIST* GENE IN X-INACTIVATION, Graeme Penny, Dominic Norris, Veronica McCabe, Emma Formstone, Alistair Newall, Sarah Duthie, Steven Sheardown, Graham Kay, Sohaila Rastan, and Neil Brockdorff, Section of Comparative Biology, MRC Clinical Sciences Centre, RPMS, Hammersmith Hospital, Du Cane Rd, London W12 ONN, UK.

X-inactivation is the dosage compensation mechanism utilized in mammals to attain equivalence of X-linked gene products between males and females. In order to understand this process we have been attempting to identify the X inactivation centre (Xic), a locus on the X chromosome that is absolutely required for X-inactivation to occur. Delineation of the Xic region on the human and mouse X chromosome has led to the identification of a candidate gene termed X inactive specific transcript (*Xist*). The *Xist* gene is expressed exclusively from the inactive X chromosome. Expression is first seen prior to the onset of X-inactivation in mouse 4-8 cell embryos and also immediately following differentiation of female embryonic stem cells (ES cells). In mouse embryos the first *Xist* expression is imprinted, correlating with the imprinted X-inactivation occuring in the first cells to differentiate from the totipotent lineage. The *Xist* gene produces a transcript of approximately 15kb which contains no significant protein coding potential and which remains localized within the nucleus, possibly in association with the Barr body. These findings have led to the suggestion that *Xist* RNA could be a cis-acting functional RNA molecule that initiates the cascade culminating in heterochromatization of the entire X chromosome. There are therefore a number of lines of circumstantial evidence that implicate *Xist* as the Xic. In order to test this hypothesis, and to prove conclusively the relevance or otherwise of Xist in X-inactivation, we have undertaken introduction of a targeted mutation into a single *Xist* allele in female ES cells. To do this we derived a female ES cell line (Pgk 12.1) carrying one X chromosome from the 129 mouse strain and the other X from the Pgk mouse strain. Polymorphisms between the two X chromosomes provides a means to assess X-linked gene expression in an allele specific manner. The X chromosomes in female ES cells are both active and when cells are allowed to differentiate in culture, random X-inactivation occurs. It is therefore possible to assess the effects of the *Xist* knockout in vitro. We have recently succeeded in generating a 7kb deletion of the *Xist* allele on the 129 derived X chromosome in Pgk 12.1 cells. Analysis of these targeted cells is currently underway and preliminary results will be presented.

B2-018 X CHROMOSOME SPECIFIC PROTEINS, CHROMATIN STRUCTURE, AND DOSAGE COMPENSATION IN *DROSOPHILA* Mitzi I. Kuroda, Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX 77030.

Dosage compensation in *Drosophila* is essential for male viability and is accomplished by increasing transcription of X-linked genes in males. Dosage compensation requires the function of at least four gene products collectively called the MSL proteins, encoded by the *maleless* and *male-specific lethal* genes (*mle*, *msl-1*, *msl-2*, and *msl-3*). Each of the four MSL proteins has been detected in a banded pattern along the length of the male X chromosome, consistent with the prediction from genetic work that they would regulate X-linked genes in males. The action of the MSL proteins is negatively regulated by the SXL protein in females, resulting in the male-specificity of dosage compensation. We have found that the MSL-1 and MSL-2 proteins are expressed in males and absent or greatly reduced in females, accounting at least in part for the sex-specificity of dosage compensation. We have found no evidence that *Sxl* controls MSL expression at the level of splicing, and are currently investigating alternative mechanisms of sex-specific regulation.

Results from several laboratories suggest that the MSL proteins act as a multimeric complex to regulate the chromatin composition of the male X. The MSL proteins co-localize on the male X chromosome, and the wild type binding pattern of each MSL protein is dependent on the function of the other *msl* genes. We have found that MSL-1 and MSL-2 co-immunoprecipitate from male larval extracts, providing biochemical support for the hypothesis that the MSL proteins interact physically. A histone H4 isoform, uniquely acetylated at lysine 16 (H4Ac16), is also preferentially associated with the male X chromosome, and its pattern of distribution is very similar to that of the MSL proteins and dependent on their function. Histone acetylation has been correlated with transcriptionally active chromatin in a number of systems. Thus, the unique chromatin composition of the male X in *Drosophila* may result in an increased accessibility of X-linked genes to the transcriptional machinery.

Control of the Expression of Sex Chromosomes (Dosage Compensation) II

B2-019 HOW FLIES MAKE ONE EQUAL TO TWO. Bruce S. Baker, Greg Bashaw, Axel Franke, Monica Gorman[1], Ignacio Marín, and Suzanne Driscoll Plump, Departments of Biological Sciences and Developmental Biology, Stanford University, Stanford, California, 94305, [1]Biology Department, University of California at San Diego, La Jolla, California, 92093.

Dosage compensation in Drosophila is achieved by the hypertranscription of the genes on the male's X chromosome. Four trans-acting factors, the products of the *msl-1, msl-2, msl-3* and *mle* genes, are known that are necessary for dosage compensation in males. Previous molecular characterization of the *mle* and *msl-1* genes (Kuroda et al., 1991, Palmer et al., 1993) showed that the MLE and MSL-1 proteins were specifically associated with hundreds of sites along the male, but not the female X chromosome, suggesting that these proteins directly mediate dosage compensation.

We have cloned the two other known *msl* genes, *msl-3* and *msl-2* . Like *mle* and *msl-1,* the *msl-3* gene is expressed in both sexes, but its protein is associated with the X chromosome only in males. Co-immunolocalization studies show that the MLE, MSL-1 and MSL-3 proteins bind to the same sites on the X chromosome. Experiments to address whether the binding of the MSL-3, MSL-1 and MLE proteins are interdependent show that the binding of all three proteins to the X chromosome require functional products from the other three *msl* genes. The *msl-3* gene from *D. virilus* has been cloned in order to compare its sequence to that of the *D. melanogaster* protein, with the goal of elucidating functionally important parts of the protein.

Since there is evidence that the *msl* genes may only mediate dosage compensation in later developmental stages we are examining the developmental time course of association of the *msl* proteins with the X chromosome during the embryonic and larval stages. In addition studies are being carried out on a mutation that may be involved in dosage compensation in the embryo.

We have also studied how dosage compensation is set in response to the X:A ratio. Previous work had shown that flies with an intermediate X:A ratio developed as mosaic intersexes with respect to sex determination, and thus sex in these individuals appeared to be an either/or decision. However studies of dosage compensation in 2X3A individuals suggested that the X chromosomes were transcribed at a rate intermediate between that of males and females in all cells. We have used antibodies to SXL and MSL proteins to show that at the level of MSL binding 2X3A individuals are also mosaic for both SXL expression and binding of MSL proteins to the X chromosome and that there is a perfect inverse correlation between SXL expression and MSL X chromosome binding.

B2-020 THE ROLE OF SXL IN THE X-CHROMOSOME BINDING AND FUNCTION OF THE MSL GENE PRODUCTS, Andres Hilfiker, Kathi McDowell, Youfeng Yang, and John Lucchesi, Emory University, Atlanta.

In Drosophila, dosage compensation, i.e. the equalization of levels of X-linked gene products in the two sexes, is achieved by the hypertranscription of most X-linked genes in males relative to females. The products of at least four genes, collectively termed male-specific lethal (*msl*) genes are required for this process and, at least in the case of three of them, mediate this function through an association with the X chromosome in males. We have found that the association of the *msl-1* and *mle* gene products with the X chromosome is negatively correlated with the level of function of the master regulatory gene *Sxl* and that it can assume either a mosaic or a uniform distribution in the tissues of mutant XX individuals. The latter is also the case in female larvae homozygous for the temperature-sensitive allele of virilizer, vir^{1ts}; however, this association does not result in a detectable level of hypertranscription nor in a reduction in viability (1). To determine if binding of the MSLs in these mutant females is due to a quantitative problem in *Sxl* or *vir* function, we are currently modulating the levels of these gene products in triploid individuals. When it can be monitored first during embryonic development, the hyperactivation of the X chromosome appears to be independent of the function of the *msl* genes although its occurrence in XX individuals is prevented by the product of the early *Sxl* promoter (2,3). In light of these observations, we are investigating when during development MSL-1 and MLE are first associated with the X chromosome in males.

(1) Hilfiker, A., et al., EMBO J., 13, 3542-3550, 1994; (2) Gergen, P., Genetics 117, 477-485, 1987; (3) Bernstein, M. and T.W. Cline, Genetics 136, 1051-1061, 1994.

Sex Determination in Other Systems

B2-021 THE GENETIC BASIS OF SEX DETERMINATION IN THE FERN, CERATOPTERIS RICHARDII. Jo Ann Banks, Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47906

In plants, the products of meiosis are haploid spores. Although homosporous ferns produce only one spore type, the haploid gametophyte derived from the spore develops as either a male or hermaphrodite. The determinant of sex type is antheridiogen (or A_{CE}), a gibberellin-like pheromone that promotes male development. In the absence of A_{CE}, individual spores develop as hermaphrodites with egg-producing archegonia, sperm-producing antheridia and a defined marginal meristem. The hermaphrodites produce and secrete A_{CE} into their surroundings yet are insensitive to its effect. In the presence of A_{CE}, spores develop as males lacking both archegonia and a defined meristem. Thus, in a population, gametophytes that germinate and develop rapidly become hermaphroditic and secrete A_{CE}, whereas those that germinate or develop more slowly become male under the influence of the pheromone. Abscisic acid (ABA) blocks the A_{CE} response in *Ceratopteris*.

In an effort to understand how A_{CE} governs the sex of the *Ceratopteris* gametophyte, several classes of mutations affecting the normal response to A_{CE} and ABA have been isolated. The *hermaphroditic* (or *her*) mutants are hermaphroditic even in the presence of A_{CE}. The *femininization* (or *fem*) mutant produces archegonia, a meristem and no antheridia. The *transformer* (or *tra*) mutant, produces only antheridia and no meristem or archegonia even in the absence of A_{CE}. The *ABA resistant* (or *abr*) mutants are hermaphroditic at concentrations of ABA that, in the wild type, suppress A_{CE}-mediated male development. By studying the epistaic interactions among these genes, a regulatory pathway of gene control affecting sex determination is proposed.

B2-022 SEX DETERMINATION IN THE SOMA AND GERM LINE OF *MUSCA DOMESTICA*, Andreas Dübendorfer[1], Denise Hilfiker-Kleiner[2], Andres Hilfiker[2], Daniel Bopp[1] and Rolf Nöthiger[1], [1]Institute of Zoology, University of Zürich-Irchel, CH-8057-Zürich, Switzerland, [2]Department of Biology, Emory University, Atlanta GA 30322.

In *Musca domestica*, presence or absence of the dominant male-determining factor *M* constitutes the primary signal for somatic sex determination. *M* acts early and transiently in embryonic development and may repress a key gene, *F*, whose activity is continuously required for female sexual development (1). A dominant factor, F^D, is epistatic over *M* and behaves like a constitutive mutation of *F* that is no longer repressible by *M* and thus dictates female development even in the presence of one or several *M* factors (2, 3).

Transplantation of pole cells (prospective germ cells) revealed that sexual differentiation of the germ cells is solely determined by the sex of the host's soma: genotypically male pole cells form functional eggs in female hosts, and genotypically female pole cells form functional sperm in male hosts. Eggs derived from *M*/+ pole cells exhibit a male-determining maternal effect so that zygotes without *M*, i.e. with a female genotype, develop as fertile males. As in the soma, F^D is also epistatic over *M* in the germ line and exhibits a maternal effect that overrules the masculinizing maternal effect of *M*, suggesting that maternal *F*-product is needed for the zygotic *F* gene to become active and to achieve female development (4). We have isolated mutations that seem to affect the funcion of *M* and *F*.

We compared *Musca* and *Drosophila*, whose sex-determining mechanisms appear different in the soma as well as in the germ line, by isolating a sequence from *Musca* cDNA with very high similarity to the *Drosophila Sxl* gene. RNA analyses performed with this fragment did not reveal any sex-specific transcripts in *Musca* so far. Antibodies against *Drosophila* SXL protein recognize a protein in *Musca* that corresponds in size to the smaller of the two isoforms expressed in *Drosophila*. In contrast to *Drosophila*, however, the same antigen is also detected in male tissues of *Musca*. In *Musca*, the homologue of *Sxl* may thus not have the same sex-determining function as *Sxl* has in *Drosophila*.

Refs. (1) Hilfiker-Kleiner et al., 1993, Genetics **134**: 1189; (2) Rubini, 1967, Genet. Agrar. **21**: 363; (3) Dübendorfer et al., 1992, Sem. Dev. Biol. **3**: 349; (4) Hilfiker-Kleiner, Dübendorfer et al., 1994, Development **120**: 2531.

B2-023 SEXUAL DIFFERENTIATION OF THE FEMALE SPOTTED HYENA (CROCUTA CROCUTA), Stephen E. Glickman[1], Christine M. Drea[1], Mary Weldele[1], Laurence G. Frank[1], Gerald Cunha[2], and Paul Licht[1], [1]University of California, Berkeley, CA 94720, [2]University of California, San Francisco.

Female spotted hyenas display the most "masculinized" external genitalia of any female mammal. There is no external vagina as the vaginal labia have fused to form a pseudoscrotum containing two bulges of fibrous/fatty tissue. The clitoris, which has hypertrophied until it is the size of the male penis, is traversed by a central urogenital canal through which the female hyena urinates, copulates and gives birth. When engaging in "meeting ceremonies" with other hyenas, females exhibit erections similar to those of the male. Internally, they are typical female mammals. Adult female spotted hyenas are also highly aggressive, somewhat larger than males and, within their multi-male, multi-female social groups, totally dominate males. According to contemporary understanding of mammalian sexual differentiation, androgens had to be circulating in female spotted hyenas during fetal life to produce the "masculinized" genitalia observed at birth. Such androgens might also have been expected to enhance the size and aggressiveness of these animals. For the past ten years, we have been studying the sexual differentiation of spotted hyenas in captive animals maintained at the Field Station for Behavioral Research in the hills adjacent to the Berkeley Campus. During this time our research group has established that, although plasma testosterone levels of female hyenas are lower than those of males, androstenedione is found in higher concentrations in females than males. This androstenedione is primarily of ovarian origin. In pregnant hyenas, androstenedione is converted to testosterone (and estrogen) by the placenta and transferred to the developing fetus. Therefore, a mechanism is in place which could account for the masculinized genitalia of the female spotted hyena. However, determining whether this is the actual route, requires interfering with the putative mechanism of androgenization. At the time of preparing this abstract, an anti-androgen (flutamide) had been administered to three pregnant female spotted hyenas, on a daily basis, beginning on days 34 - 37 of an estimated 110 day gestation. This treatment modified the external genitalia of infant male hyenas, changing morphology in the female direction, e.g., there is a larger opening at the tip of the glans penis (the urogenital meatus). The drug also modified the external genitalia of infant female hyenas, producing even larger openings at the tip of the glans clitordis --- but female hyenas were not converted to typical female mammals. The pseudoscrotum was still fused and the clitoris, although possibly reduced in length, was still enlarged and traversed by a central urogenital canal. It is possible that if we begin treatment at an earlier point in gestation and/or increase the dose of anti-androgen, more dramatic modification of the external genitalia will be observed. We are currently treating a fourth pregnant female with flutamide. In this ongoing pregnancy, we initiated administration of the drug at a considerably earlier stage of gestation. It is also possible that there is a hitherto unknown mechanism for producing male-like external genitalia and that the androgenic routes we have previously located in this species merely provide fine-tuning of the urogenital system.

B2-024 SEXUAL DIFFERENTIATION IN MARSUPIAL MAMMALS, Marilyn B. Renfree, Geoffrey Shaw, Jenny L. Harry and Deanne J. Whitworth, Department of Zoology, University of Melbourne, Parkville, Victoria, Australia.

Marsupials, like eutherians, normally require the presence of a Y chromosome for testicular formation. A male-specific homologue of the putative testis-determining gene SRY (sex-determining region of the Y chromosome) has been identified recently in marsupials. The genital ridge, which becomes the gonad, forms on the medial side of the mesonephros, the functional kidney of marsupial neonates. Germ cells migrate to the genital ridge before and during differentiation of the gonad into a testis or ovary. The testis differentiates during late fetal and early neonatal life. At day 21 of the 26.5-day gestation period in the tammar wallaby the genital ridge consists of an undifferentiated gonadal primordium. It is not until 23-24 days of gestation that the blastemal core is organized into early gonadal cords. At birth the male gonad has some of the features characteristic of a developing testis and by d2 post-partum seminiferous cords are obvious. The timing of the sequence of events is different from that of the mouse and rat. Further, in eutherians the scrotum, labia and mammary glands are sensitive to the presence or absence of testicular hormones and differentiate accordingly. In marsupials there are at least four sexually dimorphic characteristics that differentiate *before* this process of gonadal differentiation is complete, namely the scrotum, the mammary primordia, the gubernaculum and the processus vaginalis. These structures, together with the later development of the pouch, appear to be under direct genetic control, rather than secondary hormonal control as in eutherians.

We are exploiting these differences to investigate the underlying mechanisms controlling sex differentiation, at several points along the male determining pathway. First, the developmental pattern of *SRY* gene expression is different from the mouse: it is expressed for a much longer period, and as in humans is much more widespread in non-gonadal tissues. These findings for SRY raise yet another question as to its particular role(s) in sexual differentiation. Similarly Müllerian inhibiting substance (MIS) is produced over a longer time than the mouse, and is ubiquitously expressed. MIS may have direct effects on seminiferous tubule induction, as ovaries cultured with MIS or transplanted to male recipient pouch young develop tubular structures. Finally, testosterone is produced by the neonatal testis 3-4 weeks before virilization of the prostate and phallus. This virilization can be prevented by androgen receptor inhibitors, suggesting that this aspect of the sex differentiation pathway may be regulated by the androgen receptor gene. Marsupials seem destined to play a vital role in determining the modes of sex-determining genes. Clearly they have much to tell us about the evolution of mechanisms underlying these pathways in all mammals.

The Molecular Basis for Differences Between the Sexes

Evolution of Sex Determination

B2-025 EVOLUTION OF MAMMALIAN SEX CHROMOSOMES AND SEX DETERMINING GENES, Jennifer A. Marshall Graves, School of Genetics and Human Variation, La Trobe University, Melbourne, Vic 3083, Australia.

Comparisons between the three major groups of extant mammals may provide clues to the evolution and the function of mammalian sex determining systems. Eutherian ("placental"), marsupial and monotreme mammmals share an XX female:XY male chromosomal sex determining system. The X and Y, though now very different in size and gene content, must have evolved from a homologous pair early in the 200 million year history of mammals. Ancient X-Y homology is still apparent in a number of shared sequences within and outside the pseudoautosomal region, and all or most genes on the Y have partners on the X.

We have mapped a number of human X linked genes in marsupial and monotreme species. Genes on the long arm of the human X, and the proximal region of the short arm, are located on the X chromosome in marsupials and monotremes, implying that this part of the X chromosome is conserved in all mammals and must therefore represent an ancestral X. However, genes located on the remainder of the short arm of the human X map within two autosomal clusters in marsupials and monotremes. This suggests that two or more large autosomal regions were added to the X early in eutherian evolution. Since this recently added region contains X-Y shared genes, regions must have been added to the Y, as well as the X. I propose that this occurred in cycles, in which an autosomal region was added to an ancient pseudoautosomal region of a proto-X or Y, then recombined onto the other sex chromosome. Newly added regions of the Y then became subject to degradation, and the corresponding regions of the X were recruited into the X inactivation system. On this hypothesis, the pseudoautosomal region represents merely the vestige of one of these added autosomal regions, and its gene content is immaterial to its function, if any.

We have also looked for marsupial homologues of genes on the eutherian Y chromosome which act in sex determination and spermatogenesis. A homologue of the testis determining gene *SRY* is located on the Y in marsupials, and, unexpectedly, we demonstrated a *SRY*-homogous gene on the marsupial X chromosome, whose sequence is almost identical to the mouse *SOX3* gene. Sequence comparisons suggest that an ancestral *SOX* gene, perhaps with a general function in gonad development, originally had copies on both proto-X and Y chromosomes. Divergence of the X and Y may have allowed *SRY* to evolve a testis determining role, perhaps by repressing *SOX3* function. Homologues of *Ube1x/y* map also to the marsupial X and Y, but homologues of other Y-borne testis-specific eutherian genes are autosomal. Genes moved to the Y chromosome, then isolated from recombination as the X and Y differentiate, are subject to increased variation. I suggest that this results in loss of activity (and, ultimately, deletion) of most genes, but to the acquisition, by the few surviving Y-borne genes, of a male-specific function in sex determination and differentiation.

Late Abstracts

FACTORS REGULATING GERM CELL DEVELOPMENT IN THE MOUSE, Peter J. Donovan, Cell Biology of Development and Differentiation, ABL-Basic Research Program, NCI-FCRDC, Frederick, Maryland 21702.

Primordial germ cells (PGCs) are the embryonic precursor cells of the gametes of the adult animal. Mouse PGCs are first identified at day 7.0 of gestation as a small population of cells with alkaline phosphatase (AP) activity. During the subsequent 5 to 6 days of development, they migrate to the gonad analgen and proliferate rapidly to establish a population of as many as 25,000 germ cells. Shortly after arrival in the gonad PGCs stop mitotic cell division, and enter miotic arrest in the developing testis or meiosis in the ovary at day 13 of gestation. A major difference, therefore, between male and female embryos occurs in PGC development in the cessation of mitosis and entry into meiosis. By using genetic and cell culture analysis we and others have identified three growth factors required for PGC survival and proliferation: a. Steel factor (SLF, also called Stem Cell Factor, Mast Growth Factor or KL since it is the ligand for the *c-kit* tyrosine kinase receptor); b. Leukemia Inhibitory Factor (LIF); and c. basic Fibroblast Growth Factor (bFGF). The role of SLF, LIF, and bFGF (and their cognate receptors) in regulating PGC differentiation are under investigation and will be discussed.

Research sponsored by National Cancer Institute, DHHS, under the contract #NO1-CO-46000 with ABL.

THE NUCLEAR RECEPTOR SF-1 IS ESSENTIAL FOR MULTIPLE LEVELS OF REPRODUCTION Yayoi Ikeda, Xunrong Luo, Deepak S. Lala, and Keith L. Parker, Division of Endocrinology and Howard Hughes Medical Institute, Duke Univ. Medical Center Durham, NC

Steroid hormones are derived from cholesterol by the sequential action of the cytochrome P450 steroid hydroxylases. Previous promoter analyses of the mouse steroid hydroxylase genes identified a transcription factor, designated steroidogenic factor 1 (SF-1) that regulates their coordinate expression. Sequence analysis of a cDNA encoding SF-1 revealed that it is a novel member of the nuclear receptor family. *In situ* hybridization analyses of mouse embryos identified SF-1 transcripts in adrenal glands and gonads from the earliest stages of organogenesis. Interestingly, SF-1 mRNA persisted in the developing testis, but was silenced in the ovary, revealing a sexually dimorphic pattern of SF-1 expression during development. Consistent with this, promoter analyses of the gene encoding Müllerian-inhibiting Substance (MIS) strongly suggested that SF-1 regulates the expression of MIS, linking SF-1 to the regulation of both androgens and MIS, the two hormones that are required for male sexual differentiation. To address the role of SF-1 in the intact mouse, we used gene targeting to disrupt the gene encoding SF-1. SF-1 deficient animals were born at the expected frequency, indicating that SF-1 is not required for embryonic survival. However, all SF-1 deficient animals died shortly after birth due to corticosteroid deficiency. Analysis of the SF-1 deficient animals revealed agenesis of adrenal glands and gonads, selective defects in gonadotropes, and an absence of the ventromedial hypothalamic nucleus. These results establish the essential role of the gene encoding SF-1 at multiple levels of the reproductive axis.

The Molecular Basis for Differences Between the Sexes

Molecular Basis for the Primary Determination of Sex; The Functioning and Organization of the Regulatory Hierarchies that Govern Sex in Somatic Cells

B2-100 IDENTIFICATION OF X CHROMOSOME REGIONS THAT CONTAIN SEX-DETERMINATION SIGNAL ELEMENTS IN C. ELEGANS, Chantal Christ Akerib and Barbara J. Meyer, Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720.
The primary sex-determination signal of *C. elegans* is the ratio of X chromosomes to sets of autosomes (X/A ratio). This signal coordinately controls both sex determination and X-chromosome dosage compensation. To delineate regions of X that contain counted signal elements, we examined the effect on the X/A ratio of changing the dose of specific regions of X, using duplications in XO animals and deficiencies in XX animals. Based on the mutant phenotypes of genes that are controlled by the signal, we expected that increases (in males) or decreases (in hermaphrodites) in the dose of X chromosome elements could cause sex-specific lethality. We isolated duplications and deficiencies of specific X chromosome regions, using strategies that would permit their recovery regardless of whether they affect the signal. We identified a dose-sensitive region at the left end of X that contains X-chromosome signal elements. XX hermaphrodites with only one dose of this region have sex determination and dosage compensation defects, and XO males with two doses are more severely affected and die. The hermaphrodite defects are suppressed by a downstream mutation that forces all animals into the XX mode of sex determination and dosage compensation. The male lethality is suppressed by mutations that force all animals into the XO mode of both processes. We were able to subdivide this region into three smaller regions, each of which contains at least one signal element. We are pursuing several approaches to identify the signal elements in these regions. We hope to isolate mutations in signal elements as suppressors of the XO-specific lethality caused by duplications. To further map elements, we are isolating and characterizing deficiencies of the very left end of X. A duplication of this region causes reduced expression of an XO-specific reporter gene in XO animals; therefore, we predict that XX animals with a single dose of the signal element(s) in this region should have increased expression of this reporter gene. In addition, we are taking a molecular approach with one of the smaller regions that has signal element(s). In this effort we are aided by Donna Albertson (MRC, Cambridge, England), who is mapping the duplications we had isolated using in situ hybridization with YAC DNA. We are testing cosmids in this region for their ability to cause XO-specific lethality by themselves or in combination with a duplication that causes an incompletely penetrant XO-specific lethality.

B2-101 THE FUNCTION OF THE HUMAN SRY AND *MOUSE Sry GENES MAY BE DIFFERENT*, Robert A. Dubin, C. Mark Clayton and Harry Ostrer, Human Genetics Program, New York University Medical Center, New York, NY 10016
The SRY/Sry gene functions as a genetic switch in gonadal ridge initiating testis determination. The open reading frames of the human SRY and mouse Sry genes encode nuclear proteins that share a conserved 79 amino acid motif (HMG-box) that binds DNA and share no additional homology outside this region. A glutathione-S-transferase (GST)-mouse Sry HMG-box fusion protein selectively binds the sequence NACAAT from a random pool of oligonucleotides. Similar experiments with the human SRY gene are in progress, however it is known that human SRY binds AACAAT. When expressed in *HeLa cells*, the mouse *Sry gene* activates transcription of a reporter gene containing multiple copies of the AACAAT binding site. Activation was also observed for a GAL4-responsive gene when the mouse Sry ORF was linked to the DNA-binding domain of GAL4. Using this system, the activation function was mapped to a C-terminal glutamine/histidine-rich domain. When expressed in HeLa cells, the human SRY gene did not activate expression of a reporter gene containing multiple copies of AACAAT. A GAL4 DNA binding domain-human SRY fusion gene failed to activate expression, and actually reduced expression, of a GAL4-responsive reporter gene. A chimeric human SRY gene linked to the mouse Sry activation domain trans-activated through AACAAT. As a first step toward identifying proteins that interact with SRY, we have demonstrated that a GST-human SRY HMG-box fusion protein binds two HeLa proteins (approximately 92Kd and 84Kd) while GST does not.

B2-102 THE TESTIS DETERMINING FACTOR, SRY, ACTIVATES THE MULLERIAN INHIBITING SUBSTANCE PROMOTER IN A MALE RAT UROGENITAL RIDGE CELL LINE,
Christopher M. Haqq*, Tania N. Haqq*, Michael A. Weiss+ and Patricia K. Donahoe*, *Pediatric Surgical Research Laboratory and Department of Surgery, Massachusetts General Hospital, Boston, MA 02114 and +Center for Molecular Oncology and Departments of Biochemistry and Molecular Biology and of Chemistry, The University of Chicago, Chicago, IL 60637.
Mammalian sexual development is initiated in male embryos by SRY, the testis determining factor from the short arm of the Y chromosome. SRY expression in the gonadal ridge directs testicular differentiation, characterized by formation of seminiferous tubular morphology concomitant with Sertoli cell production of the Mullerian Inhibiting Substance (MIS) and Leydig cell production of testosterone. In the male, MIS regresses the female reproductive ducts, which otherwise would develop to become the uterus, fallopian tubes, and vagina. We have used the v-myc oncogene to immortalize male rat cell lines derived from 14 day embryonic urogenital ridge. In one of these line, CH34, we show that transcription of a human MIS promoter-luciferase reporter gene is regulated by cotransfection of full length human SRY. Promoter deletion analysis demonstrates the responding region to be between nucleotides -114 and -93, an element known as M2, which overlaps MIS-RE-1. SRY does not directly bind to the M2 element, suggesting the existence of an intervening SRY-induced factor or factors, designated SRYIF/s. One candidate SRYIF, Steroidogenic Factor 1 (SF1), which has been shown to upregulate the MIS promoter in postnatal Sertoli cells, was tested for ability to mediate the SRY signal in CH34 urogenital ridge cells. In CH34 cells from early gonadal development, SF1 was found to be a transcription inhibitor of the MIS promoter. When SF1 was cotransfected with SRY and MIS, SF1 abolished the SRY activity.

B2-103 CHARACTERIZATION AND EXPRESSION PROFILE OF AN UNUSUAL *Sry* TRANSCRIPT. Ross Hawkins, Robin Lovell-Badge and Blanche Capel. Department of Genetics, Cambridge University, U.K.

Characterization of the mouse *Sry* transcript has proved difficult due to a paucity of cDNA clones, absence of introns and confusing RT-PCR data. Capel et al. showed the adult testis transcript to have a novel circular structure. PCR based experiments indicate that the circle is not the major transcript in the genital ridge, the tissue where *Sry* is presumed to exert its biological effect.
In an attempt to derive an RNA-specific assay for PCR based expression analysis of *Sry*, 11.5 dpc genital ridge RNA was subjected to 3'-RACE-PCR. An *Sry* product was identified and cloned. DNA sequence analysis revealed the transcript to terminate at the 3' end of the HMG box region. Further experiments have determined that this is indeed a true, but minor, polyadenylation site.
A modified RACE primer spanning the polyadenylation site, permits the RNA-specific PCR amplification of the linear form of *Sry* transcript. Expression studies show the linear transcript to be present in the developing male gonad and adult testis. Expression of the linear form is highest in the 11.5 dpc gonad. As the gonad develops, levels of the linear and circular transcripts are essentially inversely proportional.

B2-104 NEW GENES THAT INFLUENCE THE CHOICE OF SEXUAL FATE IN C. ELEGANS, Jennifer Kopczynski and Barbara J. Meyer, Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720.

In an effort to understand how the X:A ratio determines sexual fate in the *C. elegans* embryo, it is imperative to identify and characterize the components of the X:A signal as well as the genes that transmit the signal to the downstream target genes. Towards this end, we have designed a genetic screen to identify the gene(s) that act upstream of *xol-1*, the first known gene in the sex determination pathway. *xol-1* is a switch gene that sets the choice of sexual fate in response to the X:A ratio. High *xol-1* transcript levels result in male development, while low transcript levels result in hermaphrodite development (N. Rhind *et al.*, submitted). The genetic screen we have designed utilizes a *xol-1::lacZ* reporter gene, the expression of which is also sex-specific, and thus allowed us to search directly for mutations in genes important for the sex-specific regulation of *xol-1*. This screen could identify genes that comprise the X:A ratio itself, or genes that transmit X:A signal to *xol-1*. Of 4,400 haploid genomes screened, we chose to study two X-linked mutations that have inappropriately high levels of *xol-1::lacZ* expression in XX embyros. Neither mutation strongly masculinizes hermaphrodite animals, suggesting that these new genes play either a minor or redundant role in the choice of sexual fate. Alternatively, the alleles we have in hand may not completely eliminate gene function. We are in the process of distinguishing among these possibilities by generating additional alleles of these two genes and by analyzing their phenotypes in combination with deficiencies.

A separate, but related, approach we are taking to identify the regions of the X chromosome that contain regulators of *xol-1* is to examine the affect of homozygous deficiencies on embryonic *xol-1::lacZ* expression. So far, deficiencies of the far left (*meDf6*) and far right (*mnDf41*) sides of the X chromosome result in increased *xol-1::lacZ* expression. This suggests that these regions contain genes important for the low level of *xol-1* expression in wild-type XX embryos. Consistent with this interpretation, Chantal Akerib in our laboratory has used duplications of the X chromosome to identify regions that contain signal elements, and has shown that the region uncovered by *meDf6* contains at least two elements (Genetics, in press). Thus, we believe that the *xol-1::lacZ* reporter gene will be a reliable assay to find at least some of the genes that act upstream of *xol-1* in the sex determination pathway.

B2-105 IDENTIFICATION AND MOLECULAR CHARACTERISATION OF A NOVEL GENE PROBABLY INVOLVED IN MAMMALIAN SEX DETERMINATION K.R.Rajyashri and L.Singh. Centre for Cellular and Molecular Biology, Hyderabad, 500 007 India.

Screening of human testis cDNA with a Bkm-associated human Y-specific sequence library led to the identification of a clone designated as pϕ2. pϕ2 is present in both sexes in humans and mice. It is transcribed specifically in the testis of mice. RT-PCR and RNA dot blot hybridization of pϕ2 to RNA from mouse embryos at different stages of development has revealed that it begins to be transcribed in the region of the genital ridge in 0.5 d.p.c mouse embryos and continues to be transcribed from then on in all embryonic stages. Sry, the only other gene shown to be involved in primary sex determination in mammals is also transcribed in 10.5-12.5 d.p.c mouse embryos. Like Sry, pϕ2 is also transcribed in the genital ridge of WeWe male, but not female mouse embryos. Since the WeWe embryos are devoid of germ cells, this suggests that like Sry, ϕ2 is being transcribed in the somatic cells of the male GR. In prepuberal mice, pϕ2 hybridizes with thrice the intensity to RNA from testis of newborn mice, as compared to 18 and 30 day prepuberal mice testis. Since the newborn mouse testis has three times greater proportion of Sertoli cells than the 18 and 30 day mouse testis, this suggests that pϕ2 is being transcribed in the Sertoli cells of mice testis. The similarity in the spatio-temporal pattern of expression of pϕ2 to that of Sry strongly suggests that pϕ2 has an important role in primary sex determination in mammals.

B2-106 **TRANSCRIPTIONAL REGULATION OF THE MULLERIAN INHIBITING SUBSTANCE:** W.H. Shen, Chris Moore and H.A. Ingraham. Reproductive Endocrinology Center, University of California, San Francisco, CA 94143

Expression of the testis-determining factor, SRY, leads to testes differentiation and the production of two male-specific factors, Mullerian inhibiting substance (MIS) and testosterone. Defining the molecular events initiated by SRY remains an essential question in mammalian sex determination.

We began addressing this question by studying the transcriptional regulation of the *MIS* gene. In a recent report (Shen et al., Cell 77: 651-661), we provided evidence to suggest that an evolutionarily conserved response element (CCAGGTCA) and its binding protein, the orphan nuclear receptor SF-1, are important for *MIS* gene activation. Our results implicated SF-1 as a direct or indirect target of SRY.

To assess the effect of SRY on *SF-1*, we first analyzed *MIS* and *SF-1* transcript levels by RNase protection assay during development. Interestingly, after the expression of *SRY*, *SF-1* transcript level rose in males in concordance with *MIS* transcript. The absence of SRY in females correlated with a decrease in *SF-1* transcript during Mullerian duct regression. Our observation thus linked SRY to the sexually dimorphic pattern of *SF-1* expression.

However, the difference in *SF-1* transcript levels is unlikely to be the only explanation of male-specific expression of *MIS*. Through transfection experiments in the heterologous HeLa cell line, we demonstrated that SF-1 is not constitutively active as a transcriptional factor. To activate SF-1, a ligand or a cofactor or specific modifications of SF-1 is needed. We recently identified a testicular factor, enriched in Sertoli cells, that can activate SF-1 in HeLa cells. This factor may represent another downstream target of SRY present only in the Sertoli cells. Characterization of this factor can further our understanding of the male-specific expression of *MIS* and possibly other molecular events initiated by SRY.

B2-107 CHARACTERIZATION OF THE MURINE *Zfy1* AND *Zfy2* PROMOTERS AND EXPRESSION OF A *Zfy1/lacZ* TRANSGENE IN THE SOMATIC CELLS OF THE EMBRYONIC GONAD, E.M. Simpson[&], B.P. Zambrowicz[#], J.W. Zimmermann[*], C.J. Harendza[*], S.D. Findley[#], D.C. Page[@], R.L. Brinster[*], R.D. Palmiter[#], [&]The Jackson Laboratory, Bar Harbor, ME 04609, [#]Howard Hughes Medical Institute and Dept. of Biochemistry, U. of Washington SL-15, Seattle, WA 98195, [*]Laboratory of Reproductive Physiology, School of Veterinary Medicine, U. of Pennsylvania, Philadelphia, PA 19104, [@]Howard Hughes Research Laboratories at Whitehead Institute, and Department of Biology, MIT, Cambridge MA 02142

The Y-linked mouse *Zfy1* and *Zfy2* genes, which arose by gene duplication, encode zinc-finger proteins that appear to be transcription factors. Although potential roles in sex determination and spermatogenesis have been debated, the biological functions of these genes remains unknown. *Zfy1* is expressed in ES cells, blastocysts, the embryonic gonad and adult germ cells. In contrast, *Zfy2* is not expressed in ES cells, may be expressed at very low levels in the embryonic gonad, and is expressed more abundantly than *Zfy1* in adult germ cells. To study the differential regulation of these genes, we have cloned the 5' end of the *Zfy1* mRNA, mapped the transcription start site by RT-PCR, and compared the 5' untranslated exons with those of *Zfy2*. These studies indicate that *Zfy1* and *Zfy2* use nonhomologous promoters, and this accounts for their differential regulation. The *Ube1y* gene lies about 22 kb 5' of *Zfy1* and is transcribed in the opposite direction.

Transgenes of *Zfy-1* 5' flanking DNA placed upstream of *lacZ* were constructed and characterized in ES cells and mice. Mice expressed β-galactosidase in the genital ridge of both males and females starting between embryonic day 10 (e10) and e11, peaking at e12 to e13 and then declining to low levels by e15, a pattern that matches *Zfy-1* mRNA as detected by RT-PCR. This *lacZ* expression in genital ridge was confined to somatic cells as demonstrated by its absence from the alkaline phosphatase positive germ cells. We observed normal expression of the *Zfy-1/lacZ* transgene when introduced into the *We* background, suggesting that germ cells are not necessary for this expression. In the adult, the *Zfy-1/lacZ* transgene is expressed abundantly in developing germ cells. Extragonadal (kidney, meninges, arteries, choroid plexus) expression of the transgene was also observed in embryos.

B2-108 SRY PROTEIN ENHANCES TRANSCRIPTION OF FOS-RELATED ANTIGEN 1 PROMOTER CONSTRUCTS

Andrew H. Sinclair[1], Jill McGovern[2] and Donna R. Cohen[2]

[1]Dept. of Paediatrics and Centre for Hormone Research,
The University of Melbourne,
Royal Children's Hospital, Parkville, VIC, 3052, Australia.

[2]Division of Cell Biology, John Curtin School of Medical Research,
Australian National University, Canberra, ACT, 2601, Australia.

In mammals, testis determination is under the control of the the *SRY* gene which resides on the Y chromosome. In the foetal mouse *Sry* is expressed just prior to development of the testis and shows germ-cell dependent expression in the adult mouse. SRY protein contains a high mobility group (HMG) box DNA-binding domain, and potential target sequences have been identified. The *fos*-related antigen 1 (*fra-1*) gene is closely related to the protooncogene c-*fos* and encodes a component of transcription factor AP-1. Fra-1 is expressed during spermatogenesis, and the promoter of the rat *fra-1* gene contains several potential binding sites for members of the HMG-box family of DNA-binding proteins. We demonstrate that purified SRY protein binds strongly to one of the putative *fra-1* HMG-box response elements and that SRY enhances the transcription of rat *fra-1* promoter constructs in cotransfection experiments. These results suggest that the function of HMG-box transcription factors, such as SRY, may be mediated, in part, by activation of members of the AP-1 transcription factor family.

B2-109 **Wnt-4 AS A SIGNAL REGULATING SEX DEVELOPMENT,** *Seppo J. Vainio and Andrew P. McMahon.* Department of Cellular and Molecular Biology, Harvard University, Cambridge, MA 02138. Sex determination is regulated by a series of cell-cell interactions. At the initiation it is thought that the cells from the mesonephros contribute to interstitial cells of the gonad which interact with germ cells. *Wnt-4*, a member in the *Wnt* gene family of signaling molecules is expressed in mesonephros and genital ridge at the initiation of sex development and is gradually lost during gonadal differentiation. Wnt-4 function was assayed by gene targeting to the *Wnt-4* locus. Homozygous *Wnt-4* null mice dye around 24 hr after the birth most likely because of defects in metanephros development where *Wnt-4* is also expressed. Analysis of female new born mice showed that ovary was attached to a duct with epididymis like appearance. The ovary was abnormal. Also mutant males were affected. Tubular structures possibly representing remnants of mesonephris tubules in addition to ectopic ducts were seen at the new born mice. No obvious alterations was observed in the testis. A panel of marker genes will be analyzed. Hence, Wnt-4 is involved is sex development.

The Control of Somatic Sexual Differentiation, Including Behavior

B2-200 SEX-SPECIFIC GENE EXPRESSION IN PREIMPLANTATION MOUSE EMBRYOS, Jay L. Brewster and William R. Crain, McLaughlin Institute for Biomedical Research, Great Falls, MT 59405

Mammalian embryos display sex-specific differences before implantation into the uterus. Male preimplantation embryos develop faster than female embryos and express *Sry*, the testis determining gene. The initial events in sexual differentiation may therefore occur very soon after fertilization and long before formation of the genital ridge. The focus of this study has been to identify genes in addition to *Sry* which are expressed in blastocysts in a sex dependent manner. Total nucleic acids were isolated from individual blastocysts (over 500 total) and 1/10 of the preparation used to sex the embryo by PCR detection of the Y-chromosome specific gene, *Zfy-1*. Pools of male or female lysates (30-60 embryos/pool) were then generated and the DNA removed by DNase digestion. Each pool was reverse transcribed, and used in differential display reverse transcription/ polymerase chain reaction (DDRT/PCR) analyses. Comparison of banding patterns generated from several different primer combinations revealed multiple bands which are associated only with male or female pools. Secondary confirmation using RT/PCR is being carried out on each band. Characterization of genes identified as male or female specific in early embryos should establish a framework from which the molecular basis of sex determination can be studied further.

B2-201 A MOLECULAR ANALYSIS OF THE C. ELEGANS DOSAGE COMPENSATION GENE, *DPY-21*.

Edith Cookson and Barbara Meyer, Department of Molecular and Cell Biology, University of California, Berkeley, CA.

Dosage compensation in *C. elegans* is implemented by at least five autosomal genes, *dpy-21*, *dpy-26*, *dpy-27*, *dpy-28* and *dpy-30*, whose activity states are set in response to the primary sex-determination signal, the X/A ratio. *dpy-21* is unique among the five genes in that it is not required maternally, and the wild-type protein is not essential for viability. *dpy-21* also differs from the other dosage compensation genes in that *dpy-21* XO animals have altered levels of X-linked gene expression, although they are phenotypically wild type. Analysis of a temperature-sensitive allele of *dpy-21* suggests that *dpy-21* is required in mid-embryogenesis at approximately the same stage as *dpy-27* and *dpy-28* (L. DeLong, unpubl.).

We have taken several approaches to clone *dpy-21*. A recently developed map of polymorphic sequence-tagged sites was used to localise the gene to an approximately 1Mb region on the right end of chromosome 5. Initially, cosmids and YACs from the *dpy-21* region were injected into the germline of *dpy-21* mutant animals in order to identify the gene by transformation rescue. This approach failed, due mainly to the difficulties of establishing stable transgenic lines with YAC DNA.

We subsequently narrowed the *dpy-21* region by mapping the left breakpoint of a *dpy-21* deficiency strain. RFLP mapping of animals recombinant for the two closest genetic markers has established that *dpy-21* lies on one of two YACs, which span approximately 400kb. A phage library of each YAC has been made and a contig of overlapping phage clones is being assembled by fingerprint analysis. The clones will be used refine the position of *dpy-21* by RFLP analysis. DNA from these subclones will also be used as probes to identify alterations in the genomic DNA of *dpy-21* mutant strains. Additionally, pools of phage clones will be used in transformation rescue experiments to identify which clones can complement a *dpy-21* mutant phenotype.

B2-202 EJACULATORY BULB SPECIFIC PROTEINS IN *DROSOPHILA* AND THEIR GENES - PEBmeII AND PEBmeIII. Hristem M. Dyanov, Tamara G. Bakaeva, Svetlana G. Dzitoeva and Leonid I. Korochkin, Laboratory of Developmental Genetics and Neurogenetics, Institute of Developmental Biology, RAS, Moscow, Russia.

Somatic sex-specific traits of *Drosophila melanogaster* are controlled by a hierarchy of regulatory genes, including *tra, tra2, dsx, ix, Rb97D* and others. These specify the expression of target genes in two ways: through the formation of sex-specific tissue in which the target genes are later expressed and by continuous maintenance of their expression in a sex-nonspecific tissue. Several specific proteins have been detected in the ejaculatory bulb of *Drosophila* (1,2,3). We reported the nucleotide sequences of two cDNAs, coding for two new proteins expressed exclusively in the ejaculatory bulb of *D. melanogaster* imago males - PEBme II (GenBank accessory number U08282) and PEBme III (GenBank accessory number U08281). The synthesis of both appears to be controlled by the first of the above presented mechanisms, since the corresponding mRNA expression was detected *in situ* in first 30 min after imago hatching, but not - in the pupae-stages. In this sense all members of PEB family (1,3) are suitable for study molecular and cellular mechanisms of the development. Possible functions of PEBme II and PEBme III in *Drosophila* reproduction are discussed. PEBme II is highly expressed as a heterogenous protein fraction (6-10 kDa), encoded only from one-type mRNA - probably as a result of polycistronic translation. The FASTA- and TFASTA-based similarity search against Insecta- and all GenBank data bases did not show significant homology to any known sequence. PEBme III was detected as highly expressed protein fraction (18 kDa), encoded from 480 nt mRNA. FASTA- and TFASTA- computational similarity search showed a high-scores nucleotide identity and putative amino-acid similarity with D. melanogaster CS-5 antennal-specific OS-D protein mRNA, reported as a putative pheromone-binding protein (GenBank acc. No: U02546).

References: **1.** Ludwig, M.Z., I.I.Uspensky, A.I.Ivanov, M.R.Kopantseva, Ch.M.Dyanov, N.A.Tamarina and L.I.Korochkin.1991. Biochem. Genet. 29: 215-239. **2.** Uspensky, I.I. personal communication. **3.** Sergeev, P.V., G.N. Yenikolopov, N.I. Peunova, B.A. Kuzin, R.A.Khechumian, L.I. Korochkin and G.P. Georgiev. 1993. Nucl. Ac. Res. 21: 3545-3551.

B2-203 ARE THE DROSOPHILA TRA-2 PROTEINS GENERAL SPLICING FACTORS?

William Mattox and M. Elaine McGuffin, Department of Molecular Genetics, University of Texas MD Anderson Cancer Center, Houston, TX 77030

The *tra-2* gene encodes a family of related RNA binding proteins that are known to affect the sex-specific alternative processing of pre-mRNAs from at least five different genes. These proteins appear to be expressed at all stages of development and are widely distributed in the somatic and germline tissues of both sexes. The organization of the TRA-2 proteins is very similar to that of proteins from the SR family of splicing factors which are known to play essential but sometimes functionally redundant roles during constitutive pre-mRNA splicing. This raises the issue of whether TRA-2 also performs such a general function. We have examined the distribution of TRA-2 protein molecules on nascent pre-mRNAs associated with polytene chromosomes and find that while these proteins are present at many sites the distribution of these sites is distinct from that of either a Drosophila SR protein or of Sm epitope-containing general splicing factors. Using SR depleted splicing extracts from Drosophila Schneider cells, we found that SR proteins are required for constitutive splicing in Drosophila. These extracts are complemented by addition of the human SC35 and SF2 proteins as well as by the Drosophila RBP1 protein. The TRA-2 protein, however, fails to complement these extracts indicating that it is unable to carry out the redundant essential function that is provided by other SR family proteins. Finally, deletion of a large segment of the TRA-2 protein coding sequences does not effect viability in either sex. Taken together, these results suggest that, while the TRA-2 proteins affect processing of a number of pre-mRNAs, they are not likely to have a general role in constitutive pre-mRNA splicing.

B2-204 THE PRODUCT OF THE C. ELEGANS FEM-2 GENE IS SIMILAR TO PROTEIN PHOSPHATASES FROM DIVERSE SYSTEMS. Dave Pilgrim, Angela McGregor, Petra Jäckle, Troy Johnson, and Dave Hansen, Dept. of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada.

In the sex determination system of the nematode C. elegans, the fem-2 gene acts (along with the fem-1 and -3 genes) at a branchpoint between somatic and germ line sex determination. The mutant phenotype suggests that it plays an essential role in regulating a pathway transducing a cell-nonautonomous signal (Her-1) to a nuclear transcription factor (Tra-1). The fem-2 gene was cloned based upon its genetic and physical map position. The predicted Fem-2 protein sequence is similar to protein phosphatase 2C (PP2C) enzymes from systems as diverse as yeast, humans and plants. At least one fem-2 mutant allele results from a change in a conserved amino acid. This finding implicates phosphorylation as a control mechanism in C. elegans sex determination for the first time. The protein sequence alignments suggest that Fem-2 falls into a separate class of proteins than the "classical" mammalian PP2C enzymes. Supporting this difference, we find that if the amino-terminal third of Fem-2, outside the conserved regions, is expressed in transgenic animals, a dominant feminization phenotype results. This feminization occurs in both XX and XO animals, but is restricted to the germ line, suggesting that the amino-terminus may play an important role in regulating tissue-specific Fem-2 activity.

B2-205 *tra-1* AND SEXUAL DIFFERENTIATION IN *C. elegans*, David Zarkower* and Jonathan Hodgkin
MRC Laboratory of Molecular Biology, Cambridge, England; *present address: Institute of Human Genetics, University of Minnesota Medical School, Minneapolis, MN 55455

tra-1 is the terminal global regulator of somatic sex determination, and is both necessary and sufficient to direct female somatic differentiation. The *tra-1* gene expresses two mRNAs, both encoding zinc finger proteins. TRA-1A has five zinc fingers and TRA-1B has only the first two fingers. Only TRA-1A binds DNA *in vitro*. Transgenic animals expressing each TRA-1 protein have been used to test their relative contributions to *tra-1* function. TRA-1A rescues most phenotypes of *tra-1* null mutants, while TRA-1B has no effect. Further, TRA-1A made constitutively active by a gain-of-function mutation can feminize wild type XO animals, while TRA-1B(gf) has no effect. These experiments suggest that TRA-1A has most *tra-1* activity.

The sex determination pathway resembles other signalling pathways regulating transcription factors. As some of these involve cytoplasmic sequestration and *tra-1* is regulated post-translationally, we examined the location of the TRA-1A protein, using an epitope-tagged transgene. We find that TRA-1A is predominantly nuclear at all stages in both sexes, suggesting that its down-regulation in males occurs in the nucleus.

We are interested in how *tra-1* controls the genes that carry out sexual differentiation. We are searching for genes containing potential TRA-1A binding sites, and testing whether those sites mediate regulation by *tra-1*. A number of potential targets also have been identified genetically. The current focus among these is on *mab-3*, a gene required in males to repress yolk protein expression (intestine) and to promote V-ray sensory neuron formation (tail). We are cloning *mab-3*, and have obtained phenotypic rescue with a YAC clone from the *mab-3* region.

Control of Somatic Sexual Differentiation and Sex Determination in the Germline

B2-300 TESTICULAR SECRETIONS ARE NOT SUFFICIENT TO CAUSE MASCULINE NEURAL DEVELOPMENT IN ZEBRA FINCHES, Arthur P. Arnold and Juli Wade, Dept. Physiological Science, and Brain Res. Inst., UCLA, Los Angeles CA 90024. Estrogen treatment of female zebra finches causes masculine development of the neural song system. This effect has led to the hypothesis that male neural development is caused by estrogenic metabolites of testicular secretions. However, little experimental evidence supports this idea. Plasma levels of estrogen and brain aromatase are not sexually dimorphic after hatching, and inhibition of estrogen synthesis or action after hatching does not block masculine neural development in genetic males. Here we treated zebra finch eggs on day 5 of incubation with fadrozole, a potent inhibitor of estrogen synthesis, or with saline. Birds were killed at one month after hatching. Fadrozole-treated birds possessed either bilateral testes, or mixed gonadal tissue (usually an ovotestis on the left and a testis of the right). Saline-treated birds had normal testes or ovaries. However, despite the presence of testicular tissue in every fadrozole-treated bird, there was no significant effect of treatment on morphology of brain regions in the song system (volumes of brain regions and neuronal soma sizes). Fadrozole-treated birds with ovarian tissue (probably genetic females) did not differ from saline-treated females in neural morphology, and fadrozole-treated birds with bilateral testes (probably genetic males) did not differ from saline-treated males. Other birds were similarly treated with fadrozole *in ovo* but killed in adulthood, 100 days after hatching. Fadrozole-treated birds with mixed gonads (presumed to be genetic females) invariably had female plumage, but the testicular tissue in these birds was voluminous, histologically normal, and stimulated growth of androgen-sensitive syrinx. These results indicate that the presence of a substantial amount of functional testicular tissue is not sufficient for masculine brain differentiation. Moreover, inhibition of estrogen synthesis does not block masculine neural development. The results are compatible with the idea that ovarian secretions block masculine neural development. Supported by NIH DC00217 and MH10352.

B2-301 MALE MICE HOMOZYGOUS FOR A MUTANT ALLELE OF *DESERT HEDGEHOG (DHH)* EXHIBIT EMBRYONIC AND POST-PARTUM GERM CELL DEFICIENCIES, Mark J. Bitgood, Liya Shen, and Andrew P. McMahon, Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA 02138.
We are interested in the role of peptide signals in the regulation of vertebrate development. To this end, we have identified a family of putative signalling molecules related to the *Drosophila* segment polarity gene, *hedgehog*. One of these, *desert hedgehog (Dhh)*, is expressed in the presumptive Sertoli cells soon after sexual differentiation begins and continues to be expressed in the testis into adulthood. In order to address the function of *Dhh*, we generated a presumptive null allele by homologous recombination in mouse embryo stem cells. Male mice homozygous for the allele exhibit a marked reduction in testis size and are sterile. While germ cells are present in these mice at birth, and these germ cells appear to undergo meiosis in the seminiferous tubules, homozygous males exhibit a drastic loss of germ cells during maturation of the testes. Based on these results, we postulate that *Dhh* may regulate prenatal germ cell proliferation and stem cell renewal in the male germ line.

B2-302 MAPPING AND CHARACTERISATION OF THE GENE FOR AUTOSOMAL SEX REVERSAL (SRA1) ASSOCIATED WITH CAMPOMELIC DYSPLASIA. Jamie W. Foster#, Marina A. Dominguez-Steglich#*†, Silvana Guioli, Cheni Kwok, Polly A. Weller, Jean Weissenbach¶, Sahar Mansour*§, Ian D. Young*, Peter N. Goodfellow%, J. David Brook*† and Alan J. Schafer. Department of Genetics, University of Cambridge, Downing Street, Cambridge CB2 3EH, UK. *Centre for Medical Genetics, City Hospital, and †Department of Genetics, University of Nottingham, Queens Medical Centre, Nottingham NG7 2UH, UK. ¶Societe Laboratoire Genethon, 1 Rue de l'International, Evry 91000, France. §Institute of Child Health, London, WC1N 1EH U.K.

The locus for sex reversal (SRA1) associated with campomelic dysplasia (CD) has previously been mapped to human chromosome 17q24.1-25.1 between the genes for growth hormone and thymidine kinase (Tommerup *et al.*, Nature Genetics 4: 170-174). We have constructed a radiation hybrid map across this 20 MB region using a panel of whole genome radiation fusion hybrids. Using this map we have identified DNA markers that flank the translocation breakpoint in a 46,XY,t(2;17) (q35;q23-24) female (sex reversed) CD patient (Young *et al.*, J. Med Genet 29:251-252). The flanking markers were used to identify YACs which formed the basis for a 2 Mb contig that crosses the translocation breakpoint. Further markers were isolated from this contig and a 100 kb cosmid walk was initiated which precisely mapped the breakpoint position. Adjacent to this translocation breakpoint we have identified a gene. We have characterised this gene with respect to its transcript size, sequence and the intron/exon structure. Subsequent SSCP analysis and sequencing of this gene in 9 clinically confirmed CD without cytologically detectable chromosomal rearrangements has identified two missense mutations, two frameshift mutations, one nonsense mutation and one splice mutation Three of the mutations have been confirmed to be *de novo* and three of these CD patients are XY females, proving that mutations in this gene cause both campomelic dysplasia and sex reversal.

B2-303 MUTATIONAL ANALYSIS OF A CANDIDATE GENE FOR CAMPOMELIC DYSPLASIA AND AUTOSOMAL SEX REVERSAL USING SINGLE-STRAND CONFORMATION POLYMORPHISM (SSCP)

Cheni Kwok, Polly A. Weller and Peter N. Goodfellow. Department of Genetics, University of Cambridge, Downing Street, Cambridge CB2 3EH, UK.

Single strand conformation polymorphism analysis (SSCP) is a simple and rapid method for detection of single base changes in short DNA fragments. We describe here an effective mutation screening strategy to detect mutations in *SOX 9*, a candidate gene for campomelic dysplasia (CD) and autosomal sex reversal in man.

PCR primers were designed to amplify overlapping fragments that span the entire open reading frame of *SOX 9*, including the intron-exon boundaries. PCR fragments were generated which provide a two-fold coverage of the gene for mutational screening by SSCP. Nine patients with clinically confirmed CD, of which five are also sex-reversed, were screened for mutations in *SOX 9*. We found 7 distinct mutations. These include base insertions and substitutions, including a nonsense mutation and a splice mutation. Three mutations were found to be *de novo*. We conlude that *SOX 9* is the gene responsible for both CD and autosomal sex reversal in man.

B2-304 **DIFFERENTIAL METHYLATION OF THE *Xist* GENE DURING GAMETOGENESIS CORRELATES WITH THE INHERITANCE OF IMPRINTED ALLELES IN THE MOUSE.** John McCarrey, Mira Ariel, Edward Robinson & Howard Cedar. The Southwest Foundation for Biomedical Research, San Antonio, TX 78228 and The Hebrew University, Jerusalem, Israel.

In mammals, compensation for differences between the sexes in numbers of X chromosomes is achieved by X-chromosome inactivation (XCI), such that all but one X chromosome is transcriptionally silenced in each cell. A candidate for a regulator of this process is the *Xist* gene, which maps to the X-inactivation center and is expressed exclusively from the inactivated X chromosome. Initial expression of this gene precedes XCI in both embryonic somatic cells and adult spermatogenic cells, while cessation of *Xist* expression correlates with reactivation of the previously inactive X chromosome in fetal oocytes. Both XCI and expression of *Xist* are imprinted in the early female embryo, with exclusive expression of the paternal allele of *Xist* correlating with non-random inactivation of the paternal X chromosome in extraembryonic tissues. Allele-specific methylation may play a role in the regulation of this process to mark imprinted genes in the gametes, thus providing a signal distinguishing the two parental alleles in the embryo. To evaluate imprinting of the *Xist* gene we have followed its methylation pattern througout gametogenesis and early embryogenesis. Our results indicate that CpG sites at the 5' end of the gene are methylated in the oocyte and unmethylated in sperm DNA, and this allele-specific pattern is preserved in the pre-implantation embryo. The paternal methylation pattern becomes established in prospermatogonia at about the time of birth, while the maternal pattern is already established in fetal oocytes. Thus, differential methylation represents a potential signal for initializing and maintaining allele-specific expression of the *Xist* gene, and may also represent a critical event leading to paternal-specific XCI in the extraembryonic tissues of the mouse.

B2-305 MOLECULAR GENETIC ANALYSIS OF BOYS WITH HYPOSPADIAS AND OTHER GENITAL MALFORMATIONS

Agneta Nordenskjöld, Jan Svensson and Maria Anvret, Department of Clinical Genetics and Department of Pediatric Surgery, Karolinska Hospital and St Görans Hospital, Stockholm, Sweden

Background: Genital malformation in boys can arise from different mechanisms. Because of the basal female genital differentiation, various disturbances in the male embryo during development leads to abnormal feminization of the phenotype to an extent that vary from female phenotype to isolated mild hypospadias. That can result from either insufficient hormonal stimulation from the gonad caused by dysgenesis or disturbances in the androgen metabolism.

Results: 1) DNA from 5 patients with Drash syndrome (frequent gonadal dysgenesis) was collected and directly sequenced for WT1 gene mutations. In four of the patients heterozygous point mutations in exon 8 and 9 were found. In the fifth, atypical patient, no mutation was found in the second to tenth exon. Loss of the wild type allele was shown in the three tumours from these patients.
2) DNA from 27 patients with 46 XY gonadal dysgenesis was screened for WT1 gene mutations with exon specific PCR and denaturing gradient gel electrophoresis (DGGE). One patient with a mutation in exon 8 was detected and the patient later diagnosed as having a Drash syndrome.
3). DNA from 50 individuals with a 46 XY karyotype with different genital malformations was collected. The DNA was screened for mutations with DDGE after exon-specifik amplification of the WT1, the androgene receptor and the testosteron-5-α-reductase genes.
Preliminary data suggests that WT1 gene mutations causing genital malformations are uncommon when not associated with a Wilms' tumor.

Sex Determination in Other Systems; Evolution of Sex Determination

B2-400 ANALYSIS OF GENE EXPRESSION IN THE GENITAL RIDGE OF THE CHICK EMBRYO BY *IN SITU* HYBRIDISATION

Jane E. Andrews and Andrew H. Sinclair

Dept. of Paediatrics and Centre for Hormone Research,
The University of Melbourne,
Royal Children's Hospital, Parkville, VIC, 3052, Australia.

Sex determination in vertebrates is controlled by a variety of genetic and environmental switches. Although the initiating switch may differ, the function and sequence of key regulatory genes involved in testis or ovary development are likely to be conserved throughout vertebrate evolution. This research aims to analyse the genetic mechanisms controlling Avian sex determination and elucidate general vertebrate pathways. We decided to examine embryonic chickens (*Gallus domesticus*) and generate an expression profile using genes known to be implicated in the development of the mammalian urogenital ridge. The genes chosen for study include *Aromatase, WT-1* (Wilm's Tumour 1) and *SF-1* (Steroidogenic Factor 1). Both WT-1 and SF-1 proteins appear to be required for the development of the presumptive gonad. Mice which carry mutations in either of these genes experience gonadal failure. Aromatase appears to be required for differentiation of the female phenotype. Inhibition of aromatase can cause genetic female chickens to develop as functional males. We examined expression of *WT-1, SF-1* and *Aromatase* in chick embryos by whole mount *in situ* hybridisation just before, during and after the presumptive gonad becomes sexually dimorphic.

B2-401 SEXUAL DIMORPHISM IN CORONARY AND MESENTERIC ARTERY COLLAGEN CONTENT IN RATS, D. Ely, D. Chonko, M. Karasides, M. Turner, Dept. of Biology, The University of Akron, Akron, OH 44325-3908.

Objective: To quantify the collagen network in the coronary and mesenteric arteries in four rat strains to test the hypothesis that the Y chromosome from a hypertensive father influences collagen content. Design and Methods: Collagen was measured by hydroxyproline assay (HPLC) and by computer image analysis of sections stained with Sirius red. Four strains of 4, 10 and 20 week old male and female rats were used: spontaneously hypertensive rats (SHR), normotensive Wistar Kyoto (WKY), and two hybrid crosses developed in our laboratory - SHR/a (99.9% SHR autosomal genes and the Y chromosome from a WKY) and SHR/y (99.9% WKY autosomal genes and the Y chromosome from SHR). Blood pressure was measured by tail cuff and selectively by aortic telemetry (Data Sciences). Results: All males had higher b.p. than females at 10 and 20 weeks. SHR males had significantly higher coronary and mesenteric collagen at 20 weeks as compared to females. SHR/Y males had higher b.p. (15%) and higher coronary collagen measured by HPLC (91%) and image analysis (68%) as compared to WKY at 20 weeks. SHR/a males had lower b.p. (20%) and lower coronary collagen as measured by HPLC (43%) and image analysis (41%) as compared to SHR at 20 weeks. B.P. and coronary or mesenteric collagen showed a strong correlation ($r = .76$, .82 respectively, $p < .001$). Prepubertal castration in the males significantly lowered b.p. (20-45mmHg) and coronary artery collagen (23-50%) in all SHR strains. Conclusion: The Y chromosome from SHR both raised b.p. and coronary and mesenteric artery collagen content. Males in all strains had higher b.p. than females at 20 weeks, but only in SHR strains was vessel collagen higher in males than females at 20 weeks. Testosterone is one candidate that may interact with b.p. to promote increased collagen deposition in arterial vessels.

B2-402 SEX-SPECIFIC POLYMORPHISM OF SRY AND ZFY IN THE LIZARD, *CALOTES VERSICOLOR*, WHICH LACKS SEX CHROMOSOMES AND TEMPERATURE DEPENDENT SEX DETERMINATION, S.Ganesh, J.Mohanty, and R.Raman, Cytogenetics Laboratory, Department of Zoology, Banaras Hindu University, Varanasi, India

Unlike birds and mammals where chromosomal sex determination (CSD) is well established, the group reptilia exhibits a wide variety of CSD as well as E(Environmental)SD mechanisms. Also, there are species in which neither mechanism has been demonstrated. The garden lizard, *Calotes versicolor*, is one that lacks identifiable sex chromosomes. In the present study, incubation of its eggs at low (22°C), ambient (28±2) and high (32, 35°C) temperature had no effect on the sex determination. However, exogenous application of testosterone to embryos (stage 28, 30 and 33) resulted in a majority of them developing into males. Similar treatment with estradiol had no effect on their sex. Thus, sex determination in *Calotes* could be genic; default sex being the female and heterogametic sex the male. Hence, a wild population of *Calotes* was screened with the mammalian Y-chromosomal genes, SRY and ZFY by southern hybridisation. Their distribution in male and female was as follows.

Probe	Total	Male		Female	
		+ve	-ve	+ve	-ve
SRY	67	32	00	16	19
ZFY	97	55	00	16	26

In all the positive females both the genes were present, two of which also showed RFLPs for both. In PCR amplification of the HMG box of SRY using degenerate primers, the expected fragment was detected in males as well as in the positive and "negative" females. But when probed with the SRY clone, pY53.3, the male "amplicons" hybridised much more intensely than the females' suggesting divergence of SRY sequences between the sexes.

B2-403 DOES *SRY* DETERMINE THE MARSUPIAL TESTIS?,

Jenny L. Harry[1], Peter Koopman[2], Francine E. Brennan[3], Jennifer A. Marshall Graves[3] and Marilyn B. Renfree[1], [1]Department of Zoology, University of Melbourne, Vic. 3052, [2]Centre for Molecular Biology and Biotechnology, University of Queensland, Qld. 4072, [3]Department of Genetics and Human Variation, La Trobe University, Bundoora, Vic. 3083.

There is compelling evidence that the recently isolated *SRY* gene from human and mouse is the testis determining factor on the mammalian Y chromosome. Homologues of the *SRY* gene are also localised to the Y chromosome of two unrelated marsupial species, the tammar wallaby and the Darling Downs dunnart. In the fetal mouse, *Sry* expression is confined to the genital ridge and is expressed from the appearance of the genital ridge at 10.5dpc until 12.5dpc when the Sertoli cells align into seminiferous tubules. The aim of our work was to precisely define the timing of *SRY* gene expression in relation to morphological cues during mammalian gonadogenesis. In the tammar wallaby, the genital ridge is first observed by day 21 of the 26.5 day gestation period, and it is not until 2 days post partum that the testis has an appearance similar to the 12.5dpc mouse fetal testis. We examined the developmental profile of this gene in the tammar where the equivalent stages of testicular differentiation occur over 7 days, as compared to 2 days in the mouse.

Using primers specific for tammar *SRY* gene sequence and RT-PCR analysis, we have shown that *SRY* is transcribed in the male (not female) gonad from the onset of genital ridge development until at least day 40 of pouch young development. Further, this gene is expressed in the male fetus prior to genital ridge formation (d19 being the youngest examined) and in a variety of other fetal and pouch young somatic tissues. *SRY* mRNA is also detected in a range of adult male tammar tissues. These data are very different to the expression profiles for the mouse, but are consistent with observations from humans where RT-PCR *SRY* product is not confined to presumptive and mature male gonadal tissue. There is, however, no direct evidence that *SRY* is a sex determining gene in marsupials. Characterisation of tammar *SRY* mRNA is necessary to establish if the *SRY* transcript found in the developing testis is the same as that expressed in extra-gonadal tissues.

B2-404 THE ROLE OF *SRY* GENES IN AVIAN SEX DETERMINATION

R.M. Korn, Department of Genetics, University of Cambridge, Downing Street, Cambridge, CB2 3EH UK.

The presence of the Y chromosome in mammals is the primary genetic switch leading to testis formation and thus male development. A gene from the sex determining region of the Y chromosome, *SRY*, has been shown to be the testis determining factor and is present on the Y chromosome of all mammals. No member of the family of genes related to *SRY* has been found to be uniquely situated on the avian W chromosome, which suggests that this sex determining system is not shared by the birds. Most evidence suggests that the sex of birds is determined by the Z chromosome to autosome ratio, rather than the W chromosome being a dominant inducer of femaleness. The sex of aneuploid birds with a Z0 or ZZW chromosome constitution would resolve this question. Commercial broiler chicken flocks are being screened, and results suggest the presence of Z0 females and ZZW males, supporting the genetic balance hypothesis. This screen will also identify sex-reversed birds, whose analysis could lead to the identification of novel genes involved in sex determination in birds.

B2-405 SEX SPECIFIC TRANSCRIPTS FROM CHICK GENITAL RIDGE IDENTIFIED BY DIFFERENTIAL DISPLAY RT-PCR.

Michael J. O'Neill, Michele Binder and Andrew H. Sinclair

Dept. of Paediatrics and Centre for Hormone Research,
The University of Melbourne,
Royal Children's Hospital, Parkville, VIC, 3052, Australia.

The mammalian Y-linked testis determining gene *SRY* acts on the sex determining pathway and induces the indifferent embryonic gonad to develop as a testis. Sexual differentiation in other vertebrates is controlled by a variety of genetic and environmental switches. Although these switch mechanisms vary, the critical steps leading to testis or ovary development are likely to be controlled by key regulatory genes whose function and sequence have been conserved throughout evolution. We aim to elucidate the genetic mechanisms controlling Avian sex determination, which may also help us to better understand mammalian sex determination. As little is known about Avian sex determining genes we decided to search for sex specific transcripts from the developing urogenital ridge of embryonic chickens (*Gallus domesticus*). We examined transcripts just before (4.5 days), during (5.5d) and after (6.5d) the presumptive gonad becomes sexually dimorphic. We employed RNA fingerprinting or Differential Display RT-PCR (DDRT-PCR) to identify sex specific transcripts. Refinement of the technique has allowed us to display > 40,000 partial cDNA's, the vast majority of which are common to both males and females. However, we have isolated and cloned several sex specific transcripts. Sex specific expression was confirmed by hybridization to mini-Northern blots. To obtain full-length cDNA clones we pooled the sex specific DDRT-PCR cDNA clones and used them to screen male and female genital ridge cDNA libraries.

B2-406 THE ROLE OF p450 AROMATASE IN TEMPERATURE DEPENDENT SEX DETERMINATION IN REPTILES.

Jeyasuria Pancharatnam[1], Allen R. Place[1], Valentine A. Lance[2] and Bruce Blumberg[3]. Center of Marine Biotechnology, Maryland Biotechnology Institute, University of Maryland, Baltimore MD,[1]. Center for Reproduction in Endangered Species, Zoological Society of San Diego[2], Salk Institute, La Jolla, CA[3].

In reptiles, the primary switch that determines male versus female pathway during development may be genotypically (GSD) or environmentally (ESD) triggered. In temperature dependent sex determination (TSD), the sex of the individual is determined by the temperature at which the eggs are incubated during the temperature sensitive period. The temperature sensitive period is generally in the middle third of incubation. We have shown that eggs of *Malaclemys terrapin* (the diamondback terrapin) incubated at low temperatures (<28°C) produce males, while high temperatures (>30°C) produce females. Alligators (*Alligator mississippiensis*), on the other hand, produce females at 29-31°C, males at 33°C, and females again at 35°C.

Since embryos of most reptiles incubated at male producing temperatures can be feminized by the application of estrogen, it has been proposed that estrogen synthesis triggers the sex determinative process in reptiles. We have therefore cloned the coding sequence for aromatase p450 from a terrapin ovary cDNA library and the aromatase p450 from an alligator ovary cDNA library in order to investigate the role of this enzyme during development. To date, the entire coding sequence of the terrapin aromatase p450 and a portion of the 3' end of the alligator aromatase p450 have been determined. The aromatase sequences of terrapin and alligator show more similarity with chicken aromatase p450 than the equivalent sequences in mammals and fish.

B2-407 THE ISOLATION OF SEX-SPECIFIC AND SEX DETERMINATION GENE SEQUENCES OF THE QUEENSLAND FRUIT FLY, *Bactrocera tryoni*. Deborah C. A. Shearman, John A. Sved and Marianne Frommer, School of Biological Sciences, University of Sydney, NSW, Australia 2006.

Bactrocera tryoni, (formerly *Dacus tryoni*, family Tephritidae) is a serious horticultural pest in Australia and methods of biological control, such as sterile insect release (SIT), are being researched. The success of these methods relies in part on a knowledge of the genetics of the organism and an ability to manipulate the genome including sex-specific gene expression. Unlike other dipteran species, such as *Drosophila melanogaster, Musca domestica, Lucilia cuprina* and a closer relative, *Ceratitis capitata*, the genetics of *B. tryoni* have not been well studied. Sex in *B. tryoni* is suspected to be Y determined as is the case in *C. capitata*.

Two approaches have been taken in the isolation of both sex-specific sequences of and portions of sex determination genes. Degenerate PCR primers were designed to regions of greatest identity between the chorion gene of *D. melanogaster*, *D. virilis* and *C. capitata*. A 262bp product was isolated, cloned and sequenced. Translation of this region shows a high degree of identity between *B. tryoni* and *C. capitata* with no amino acid differences. Degenerate primers were also designed to the DNA binding domain of the *Drosophila doublesex* gene. PCR products are currently being analysed.

The second approach uses the RAPD technique with 10mer primers and extracts of male only and female only genomic DNA. An intense band of 420bp which was amplified only from male DNA proved to be more abundant in the male genome but not male-specific. Other potential male-specific sequences are in the process of being cloned and sequenced.

B2-408 **EXPRESSION OF *SRY*-RELATED SEQUENCES (*MDSOX*) IN TELEOST FISH, MEDAKA (*ORYZIAS LATIPES*)**, Minoru Tanaka[1], Sachiko Fukada[1] and Yoshitaka Nagahama, Lab. of Reproductive Biology, National Institute for Basic Biology, Okazaki 444, Japan

Sex in medaka (*Oryzias latipes*, strain d-RR and Hd-rR) is genetically fixed but their phenotypic sex can be easily converted by administration of steroid hormones. On the other hand mammalian phenotypic sex has to be rigidly determined in 1:1 ratio in highly estrogenic enviroment in uterus. Therefore medaka offers a good model to investigate the phylogenetical relationship between the involvement of steroid hormones in sex determination and *Sry*-related gene function. As one of the first steps to access the problem we determined several kinds of HMG regions of medaka genomic *Sry*-related sequences. They can be divided into two groups (*mdksox1* and *mdksox2* groups) based on amino acid homology with those of mice. Interestingly, two marsupial *Sry*-like sequences are more closely related to medaka *Sox* sequences than to the other mammalian *Sry* in the HMG regions. Furthermore we have isolated cDNA sequences from medaka embryos. *Mdksox2A* and *2B* is predicted to encode a polypeptide highly homologous to mouse SOX 2 and 3, respectively. The transcripts are maternally detected in both matured and ovulated oocytes. After fertilization the expression begins to be restricted to germ ring and epiblasts in embryonic shield in early embryogenesis, and detected in germ cells and neuroblasts in late embryogenesis by *in situ* hybridization. Although *mdksox* are present on both male and female genomes, mapping of *mdksox* to medaka chromosomes is now in progress to provide with data on evolution of Sry-related sequence on sex chromosomes.

B2-409 DIVERGENCE IN THE HMG REGION OF THE SRY LOCUS COMPARED TO OTHER REGIONS OF THE RAT Y CHROMOSOME, M. Turner, E. Graham, A. Qadri, M. Montgomery, D. Ely and Amy Milstead, Department of Biology, University of Akron, Akron, OH44325-3908

The Y chromosome of the Spontaneously Hypertensive Rat (SHR) contains loci which increase blood pressure and affect the timing of testosterone during puberty. To identify and compare DNA markers from hypertensive (SHR) and normotensive (WKY) rat Y chromosomes, sequence tagged sites specific for human Y chromosome were used to amplify rat DNA under permissive conditions. Amplification patterns for these primers were compared in SHR and WKY males and females. Nine of the human Y chromosome markers identify male specific bands in either the SHR, WKY or both rat strains. Five of the 9 sequence tagged sites identify male specific bands polymorphic between SHR and WKY. From a partial sequence of the rat *Sry* locus primers within the HMG region were chosen. These primers amplify a 258bp region of the *Sry* locus from male DNA only. The entire region between the primers (213bp) was sequenced.The sequence comparison of the HMG region of *Sry* identifies no sequence divergence between SHR and WKY in the 213 bases compared. Selective constraints on the HMG region are most likely responsible for the reduced variation in this region. We have previously measured divergence in these strains for both nuclear DNA and mitochondrial DNA (mtDNA). Neither mtDNA or the HMG region of the *Sry* locus have any divergence between these strains. The levels of nuclear DNA and Y chromosome divergence are much greater than would be predicted from the known origin of the strains.

B2-410 CHROMOSOMAL AND TEMPERATURE-DEPENDENT SEX DETERMINATION IN GECKO LIZARDS. Elizabeth M.A. Valleley, Jonathan Adams & P. Louise Coletta, Molecular Medicine Unit, Clinical Sciences Building, St. James's University Hospital, Leeds, LS9 7TF, UK.

The sex-determining systems employed by vertebrates are surprisingly diverse, and at first sight there appears to be no common mechanism. In mammals and birds chromosomal sex determination (CSD) is found, where the sex of offspring is fixed by the inheritance of heteromorphic sex chromosomes. In mammals the male is the heterogametic sex (XY-male; XX-female), whereas in birds the female is heterogametic (ZW-female; ZZ-male). In reptiles a wide variety of systems occur, including male and female heterogamety, parthenogensis and temperature-dependent sex determination (TSD). In species with TSD, sex is determined by the incubation temperature of the egg during embryonic development.

The evolutionary and molecular relationship between the different types of sex-determining mechanisms is unknown. Evidence suggests that the underlying mechanisms of TSD and CSD are fundamentally linked. Examples of both systems can be found in lizard and turtle species of the same genus, suggesting that there could be a rapid evolutionary transition from one system to the other.

We are investigating the molecular mechanisms of sex determination by comparing two related species of gecko lizards: the Tokay gecko (*Gekko gecko*) which has recently-evolved sex chromosomes, and the Leopard gecko (*Eublepharis macularius*) with TSD. Since the discovery of the mammalian sex-determining gene, *SRY*, homologous sex-determining genes have not been identified in reptiles or birds. We are using a different approach to identifying putative reptilian sex-determining genes by trying to isolate genes which are present on the Tokay gecko Y chromosome. Any such genes may be conserved in the Leopard gecko, and may function in TSD.

B2-411 SEX DIFFERENCES IN RECOMBINATION FREQUENCY BETWEEN SEX CHROMOSOMES IN A FISH, THE JAPANESE MEDAKA (*ORYZIAS LATIPES*) H. Wada, K. Naruse, A. Shimada and A. Shima, Laboratory of Radiation Biology, School of Science, University of Tokyo, Tokyo 113, Japan.

In a fish, the Japanese Medaka (*Oryzias latipes*), the stable sex determination with male heterogamety (XY/XX type) was demonstrated through the inheritance of a sex-linked color locus (Aida, 1921). Although the sex determination is stable, the sex chromosomes of this fish are considered to be an undifferentiated primitive state, because their sex chromosomes are morphologically indistinguishable, dominant coloration genes are located on the Y chromosome, and recombinations between X and Y chromosomes were demonstrated (Yamamoto, 1964).

To clarify the recombination pattern all over the sex chromosomes, we used three Medaka inbred strains, HNI, Hd-rR and AA2 for backcrossing to detect RAPD (Random Amplified Polymorphic DNA, Williams *et. al.*, 1990) markers. We have compared between males (XY) and females (XX) the recombination frequencies among multiple loci including 7 RAPD markers and two visible mutant loci (*r*, colorless xanthophore and *lf*, leucophore-free, both of which are coloration genes.) on the sex chromosomes. In the two independent backcrosses, Hd-rR X (Hd-rR X HNI) and AA2 X (AA2 X HNI), remarkably restricted recombinations between sex chromosomes in males (XY) were found in such region as near the sex determining locus and containing the two coloration loci.

Restrictions of recombinations are considered to be important steps for Y (or W) chromosomes to lose their genetic activities (Rice, 1987, 1994, Charlesworth, 1991). Our results support that recombination restriction can be achieved prior to the differentiations of sex chromosomes. The Medaka should be a useful model to explore a primitive stage in the evolution of sex chromosomes in vertebrates.

Late Abstracts

SRY-LIKE GENES AND TEMPERATURE DEPENDENT SEX-DETERMINATION IN THE AMERICAN ALLIGATOR. Colette M. Johnston and Paul T. Sharpe, Dept. of Craniofacial Development, Guy's Hospital, London SE1 9RT, UK.

The American alligator, *Alligator mississippiensis*, does not possess sex chromosomes and its sex is determined by incubation temperature during embryogenesis. In mammals, SRY (sex determining region of the Y chromosome) has been identified as a key regulatory gene in sex determination. In the search for a universal sex determining mechanism, a number of genes were isolated from chicken which possessed a similar sequence to the DNA binding motif of SRY. These were named Sox genes (SRY like box). Of this gene family, Sox3 is the most closely related to SRY: the high sequence similarity within the DNA binding motif suggests functional conservation. In mammals and marsupials Sox3 is located on the X chromosome.

We have identified a sequence from the alligator genome that shares strong identity with the mouse, human and chicken Sox3 genes. The nucleotide sequence of alligator Sox3 suggests that the function is conserved between mammals, birds and reptiles. We are currently determining the expression pattern and function of Sox3 in *A. mississippiensis* and investigating the potential role of Sox3 in temperature dependent sex determination.

mRNA DIFFERENTIAL DISPLAY AS A WAY OF FINDING NOVEL AND SEX SPECIFIC GENES IN THE DEVELOPING GONAD

Katarina Nordqvist[1] and Robin Lovell-Badge[2]. Dept. Cell and Molecular Biology, Karolinska Institute, 171 77 Stockholm, Sweden[1]. Developmental Genetics, National Institute for Medical Research, MRC Mill Hill, London, UK[2].

One of the more successful ways of finding genes important for sexual development has been through the study of humans showing different types of sex reversal. *SRY*, *Wt-1* and the majority of the steroid hydroxylase genes were identified in this way. However, there still exist some novel genes to hunt for.

Another way of finding novel genes important for sex differentiation is to compare mRNA populations from developing male and female gonads and we have chosen to use the mRNA differential display technique (P. Liang and A. Pardee, 1992, Science 257, 967-971). The basic principle with this technique is to display a subset of mRNAs with help of reverse transcription and polymerase chain reaction (PCR). By comparing cDNA populations from developing female and male genital ridges, it is possible to identify both female and male specific cDNAs at the same time. mRNA from 13.5 dpc male and female genital ridges have been used in the mRNA differential display. The reasons for use of this time point are several; at this time during development of the gonads it is easy to distinguish between male and female gonad. Thus, contamination of female mRNAs in the male mRNA population, and vice versa, will be reduced to a minimum. Also, at 13.5 dpc, the male gonad has been developing for 1-2 days and genes involved in this process will hopefully be expressed (as for *Wt-1* and *Ftz-F1*). The first event of female development, germ cells entering meiosis, has also started and this gives us the possibility to isolate genes important for organogensis of the ovary.

Up to date, we have cloned four sex specific cDNA fragments. Two of these, both which were isolated from the male specific mRNA differential display, show perfect homology with two steroid hydroxylase genes when compared with the EMBL data base. These results show that the differential display method is working for isolating at least male but probably also female specific genes expressed in developing gonads.

Several genes as *Sry*, *Wt-1*, *MIS* and *Ftz-F1* are, in addition to their foetal expression, also expressed in adult testis. Based on this fact, we have developed a second approach to isolate male specific genes involved in gonad development. In collaboration with Dr. Christer Hoog, Karolinska Institute, Stockholm, who has isolated over 250 different adult testis specific clones, we are currently investigating if some of these clones show foetal expression. Up to date, we have found one cDNA, CH 67, which shows an interesting expression pattern in foetal gonads.