For the sixth time in our short website history, we are undertaking the task of presenting a brief report of an Annual Meeting of the Endocrine Society, presently the 93rd, which convened at the Convention and Exhibition Center in Boston, MA, June 4-7, 2011.

As in previous similar events, the Annual Meeting Steering Committee of the Endocrine Society designed an education program worthy of a meeting known as one of the highest quality endocrinology events in the world. Indeed, they put together a line-up featuring 16 Plenary Lectures, 81 Symposia, 7 Special Sessions, 5 Endocrine “Year in” Sessions, 16 Case Management Forums, 53 Meet-the-Professor sessions, Endocrine Debate sessions, and Practice Management and Career Development workshops. In all, ENDO 2011 features more than 350 presentations from the world’s leaders in endocrine research and care (ENDO daily). Furthermore, 2589 Free Communications were accepted, 270 as Oral Presentation and 2319 as Poster Presentation. At any given time, there were multiple events happening simultaneously. It was a feast of information and offered something for everyone. As always the Plenary Lectures were the cornerstone of the meeting, with top-quality speakers and great breadth of topics.

As usual, The Exhibit Floor offered numerous booths with diverse materials of interest for the more than 7800 scientific attendees. Additional information can be gathered at the web site of the Endocrine Society, "www.endo-society.org".

Abstracts and Comments on some of the Plenary Lectures and Symposia follow. In some cases, no abstract or a very short one is published after the announcement of the Conferences, therefore, comments selected from recent articles by the lecturer are then posted.


RM Evans. Salk Institute/Howard Hughes Medical Institute, San Diego, CA.

Nuclear receptors are ligand dependent transcription factors that play fundamental roles in organ physiology by regulating the activity of complex gene networks. They act through a common mechanistic template by recruiting chromatin modifying factors including repressors and activators and use this shared template to achieve genomic integration. Through this process the 48 human receptors control metabolic homeostasis, inflammation, reproduction, repair and cell growth. They are regulatory targets for classic nuclear active hormonal lipids including steroids, retinoids, vitamin D and thyroid hormone. The orphan branch of the family are controlled by non-classic nuclear hormonal lipids such as fatty acids, bile acids, cholesterol and lipophilic xenobiotic compounds.

The presentation will address key aspects as to how dynamic regulation of complex genomic networks is achieved in the context of circadian rhythm, diabetes, inflammation and cancer. In particular, nuclear receptor regulation of the balance between glycolytic and oxidative metabolism plays a key role in obesity, a primary risk factor for insulin resistance, hyperlipidemia, hypertension and heart disease. Exercise is
a known beneficial factor in many diseases, and we have discovered that certain nuclear receptors active drugs can confer the benefits of exercise even in sedentary animals. The use of these 'exercise mimetics' to treat cardiovascular disease, frailty and insulin resistance will be discussed.

[1.1-3] Presidential Plenary Lecture: Nuclear Receptor Coactivators: Masters of Physiology & Disease
BW O’Malley. Baylor College of Medicine, Houston, TX.
Extracts from a recent publication by the speaker:
Biochemistry. 2011, 50, 313–328. Nuclear Receptor Coactivators: Structural and Functional Biochemistry. Bulynko YA, O’Malley BW. Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX, USA.
Abstract: Transcription of eukaryotic cell is a multistep process tightly controlled by concerted action of macromolecules. Nuclear receptors are ligand-activated sequence-specific transcription factors that bind DNA and activate (or repress) transcription of specific sets of nuclear target genes. Successful activation of transcription by nuclear receptors and most other transcription factors requires “coregulators” of transcription. Coregulators make up a diverse family of proteins that physically interact with and modulate the activity of transcription factors and other components of the gene expression machinery via multiple biochemical mechanisms. The coregulators include coactivators that accomplish reactions required for activation of transcription and corepressors that suppress transcription. This review summarizes our current knowledge of nuclear receptor coactivators with an emphasis on their biochemical mechanisms of action and means of regulation.

Introduction. Eukaryotic transcription is a tightly controlled multistep process that involves ordered action of protein macromolecules and their conglomerates, acting as multisubunit complexes. The precision and processivity of each step of transcription; from transcript initiation, elongation, splicing, and termination to maturation and export from the nucleus; is ensured by concerted actions of specific sets of such complexes. Typically, initiation of gene expression in the context of a eukaryotic nucleus requires recognition of specific DNA sequences by a diverse class of protein molecules, sequence-specific transcription factors (TFs), which upon binding to DNA recruit chromatin-remodeling protein complexes to “free” DNA from its tightly chromatinized state and to enable stable interactions with the general transcriptional machinery (GTFs) and RNA polymerase. Transcription initiation, elongation, RNA splicing, and transcription termination are controlled by separate specific sets of factors. Nuclear receptors (NR) comprise a large family of transcription factors characterized by similarity in their modular structure and consisting of DNA-binding and ligand-binding domains. With few exceptions, acquisition of ligand by the ligand-binding domain causes conformational change leading to dimerization, nuclear import, and binding of nuclear receptors to specific DNA sequences. This conformational change exposes interaction surfaces for recruitment of transcription accessory factors termed coregulators of transcription (CoRegs), without which the transcription factors are unable to efficiently initiate gene expression. In addition to coactivators that enhance transcription, the coregulator family includes corepressors that repress transcription. Thus, two opposing molecular forces emerge as absolute requirements for accurate and efficient regulation of eukaryotic gene expression. Most known NR CoRegs function with other transcription factors as well, indicating their universal requirement for successful gene expression. This review will focus primarily on coactivators, their biochemical and structural properties, and molecular mechanisms for regulation of their functions.

Concluding Remarks. More than a decade of studies of nuclear receptor coregulators (coactivators and corepressors) have led to the discovery of more than 350 proteins with transcriptional regulation potential, and this list continues to grow (http://www.nursa.org). The diversity in structure and biochemical functions of NR coactivators reflects the complexity of transcriptional control and highlights the ability of coactivators to regulate transcription at all stages. Such multifunctionality is achieved at least in part through assembly of coactivators into multisubunit protein complexes.
The intersubunit interactions within these complexes and their composition and associations with NR and general transcriptional machinery are regulated through the management of coactivator levels and posttranslational modifications. Coactivator concentrations in the cell are highly controlled through coactivator gene transcription, RNA translation, and protein stability. Each coactivator has several mechanisms of regulation, and the multitude of posttranslational protein modifications play a very important part by altering coactivator localization, stability, or protein-protein interactions. Moreover, posttranslational protein modifications (PTMs) also serve to transduce physiological information to coactivator molecules through the establishment of specific PTM landscapes on coactivators as a result of environmental signal-specific cascades. Diverse exogenous stimuli initiate specific kinase-driven phosphorylation pathways that trigger further PTMs, including acetylation, methylation, and ubiquitination and resulting in a complex signal-dependent PTM “coding” of coactivators, which in turn drive signal dependent coactivator functions. Because one coactivator can participate in multiple transcriptional processes (including extranuclear activities), such control ensures coordination of multiple steps required in the global response to exogenous stimuli. Thus, coactivators serve as “hubs” for physiological regulation of transcription by coordinating intracellular signaling cascades. Further research is needed to delineate specific cross-talk between PTMs and other cellular signaling pathways and to determine the roles of the multitude of PTMs identified to date on coactivators. Understanding this cross-talk is particularly important in pathologies such as cancer, in which cross-activation of kinase cascade signaling leads to an outgrowth of cells that bypass conventional, one-pathway-based therapy.

LJ Guillette. Medical University of South Carolina, Charleston, SC.


Department of Obstetrics and Gynecology, Medical University of South Carolina, Charleston, USA.

Abstract. Environmental contaminants are now a ubiquitous part of the ecological landscape, and a growing literature describes the ability of many of these chemicals to alter the developmental trajectory of the embryo. Because many environmental pollutants readily bioaccumulate in lipid rich tissues, wildlife can attain considerable body burdens. Embryos are often exposed to these pollutants through maternal transfer, and a growing number of studies report long-term or permanent developmental consequences. Many biological mechanisms are reportedly affected by environmental contaminants in the developing embryo and fetus, including neurodevelopment, steroidogenesis, gonadal differentiation, and liver function. Embryos are not exposed to one chemical at a time, but are chronically exposed to many chemicals simultaneously. Mixture studies show that for some developmental disorders, mixtures of chemicals cause a more deleterious response than would be predicted from their individual toxicities. Synergistic responses to low dose mixtures make it difficult to estimate developmental outcomes, and as such, traditional toxicity testing often results in an underestimate of exposure risks. In addition, the knowledge that biological systems do not necessarily respond in a dose-dependent fashion, and that very low doses of a chemical can prove more harmful than higher doses, has created a paradigm shift in studies of environmental contaminant-induced dysfunction. Although laboratory studies are critical for providing dose-response relationships and determining specific mechanisms involved in disease etiology, wildlife sentinels more accurately reflect the genetic diversity of real world exposure conditions, and continue to alert scientists and health professionals alike of the consequences of developmental exposures to environmental pollutants.

[L2-2] Sex Chromosome Evolution & Medicine
DC Page. Massachusetts Institute of Technology/Howard Hughes Medical Institute,
Cambridge, MA.
In recent years, genomic analysis has revealed that the human X and Y chromosomes evolved from a pair of chromosomes – autosomes – that were identical in males and females of our reptilian ancestors. I will describe how these evolutionary and genomic perspectives provide insights into the roles of the X and Y chromosomes in health and disease, including sex differentiation, male infertility, and Turner syndrome. I will also discuss prominent predictions of the human Y chromosome's imminent demise.

Extracts from a recent publication by the speaker:
Proc Natl Acad Sci U S A. 2011 108:7443-8. Licensing of gametogenesis, dependent on RNA binding protein DAZL, as a gateway to sexual differentiation of fetal germ cells. Gill ME, Hu YC, Lin Y, Page DC. Howard Hughes Medical Institute, Whitehead Institute, and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA, USA.

Abstract. Mammalian oocytes and spermatozoa derive from fetal cells shared by the sexes. These primordial germ cells (PGCs) migrate to the developing somatic gonad, giving rise to oocytes or spermatozoa. These opposing sexual fates are determined not by the PGCs' own sex chromosome constitution (XX or XY), but by the sexual identity of the fetal gonad that they enter. We asked whether PGCs undergo a developmental transition that enables them to respond to feminizing or masculinizing cues from fetal ovary or testis. We conducted in vivo genetic studies of DAZL, an RNA-binding protein expressed in both ovarian and testicular germ cells. We found that germ cells in C57BL/6 Dazl-deficient fetuses--whether XX or XY--migrate to the gonad but do not develop either male or female features. Instead, they remain in a sexually undifferentiated state similar to that of migrating PGCs. Thus, germ cells in C57BL/6 Dazl-deficient fetuses do not respond to sexual cues from ovary or testis, whereas the earlier processes of germ cell specification and migration are unaffected. We propose that PGCs of both XX and XY fetuses undergo licensing, an active developmental transition that enables the resultant gametogenesis-competent cells to respond to feminizing or masculinizing cues produced by the fetal ovary or testis and hence to embark on oogenesis or spermatogenesis. In C57BL/6 mice, DAZL is required for licensing. Licensing serves as a gateway from the embryonic processes shared between the sexes--germ cell specification and migration--to the sex-specific pathways of oogenesis and spermatogenesis.

[L3-1] Gerald D Aurbach Award Lecture: Obesity, Type 2 Diabetes & Cancer: The Insulin & Insulin-Like Growth Factor Connection
D LeRoith. Mount Sinai School of Medicine, New York, NY.
Recent studies have demonstrated a role for both the insulin receptor (IR) and the IGF-1 receptor (IGF-1R) in cancer development, growth and cancer-related mortality. Epidemiological studies showed a relationship between total IGF-1 circulating levels and the relative risk of developing most of the common epithelial cancers including prostate, colon, and breast cancer. Tissue and cell culture studies have shown an increased expression of the IGF-1R in cancer cells. The increased gene expression is the result of both enhanced promoter activity and increased translation. The formed is seen in many cancer cells that express mutated tumor suppressor gene products such as p53, WT1 and BRACA1/2. Enhanced translation is seen with deletion of PTEN, another common tumor suppressor that is mutated in cancers. Blocking the activation of the IGF-1R inhibits cancer growth both in vitro and in vivo. This has led to the development of numerous humanized IGF-1R blocking antibodies and a number of tyrosine kinase inhibitors (TKI), that have entered various phases of preclinical and clinical testing in various epithelial cancers and sarcomas.

On the hand, interest has also focused on the role of insulin in promoting cancer in obesity and Type 2 diabetes (T2D). Again, interest has arisen from epidemiological studies that find an association between C-peptide and serum insulin levels and cancer risk and cancer-related mortality in obesity and/or T2D. In the case of breast cancer, studies have convincingly shown that prognosis is worse when the breast cancer samples express higher levels of IR that is activated. In these case the expression of IR-A, a mitogenic sub-type of the IR is also expressed at high levels. To demonstrate the
direct causality between endogenous hyperinsulinemia and cancer growth and metastases, we have utilized such a mouse model. Our results show that reducing the hyperinsulinemia or blocking the IR/IGF-1R tyrosine kinases blocks the extra growth of the tumors considered secondary to the hyperinsulinemia. Thus the insulin and IGF-1 systems are involved in cancer growth and development and afford a novel new adjunct to conventional chemotherapy.

[1.3-2] Clinical Investigator Award Lecture: From Base Change to Better Care in Neonatal Diabetes
A Hattersley. Peninsula Medical School, Exeter, UK.
Extracts from a recent publication by the speaker:
Abstract. Heterozygous coding mutations in the INS gene that encodes preproinsulin were recently shown to be an important cause of permanent neonatal diabetes. These dominantly acting mutations prevent normal folding of proinsulin, which leads to beta-cell death through endoplasmic reticulum stress and apoptosis. We now report 10 different recessive INS mutations in 15 probands with neonatal diabetes. Functional studies showed that recessive mutations resulted in diabetes because of decreased insulin biosynthesis through distinct mechanisms, including gene deletion, lack of the translation initiation signal, and altered mRNA stability because of the disruption of a polyadenylation signal. A subset of recessive mutations caused abnormal INS transcription, including the deletion of the C1 and E1 cis regulatory elements, or three different single base-pair substitutions in a CC dinucleotide sequence located between E1 and A1 elements. In keeping with an earlier and more severe beta-cell defect, patients with recessive INS mutations had a lower birth weight (-3.2 SD score vs. -2.0 SD score) and were diagnosed earlier (median 1 week vs. 10 weeks) compared to those with dominant INS mutations. Mutations in the insulin gene can therefore result in neonatal diabetes as a result of two contrasting pathogenic mechanisms. Moreover, the recessively inherited mutations provide a genetic demonstration of the essential role of multiple sequence elements that regulate the biosynthesis of insulin in man.
Introduction. Neonatal diabetes is diagnosed within the first 6 months of life and there are two main clinical subtypes: the persistent, permanent neonatal diabetes (PNDM) and the remitting and frequently relapsing, transient neonatal diabetes (TNDM). Recently there have been considerable advances in the understanding of the genetics of neonatal diabetes. Most patients with PNDM have activating mutations in KCNJ11 or ABCC8, the genes encoding the potassium ATP-sensitive (K<sub>ATP</sub>) channel subunits Kir6.2 and SUR1, or heterozygous mutations in the preproinsulin (INS) gene. In contrast, abnormalities in chromosome 6q24 are the most common cause of TNDM, followed by mutations in the KCNJ11 and ABCC8 genes. Despite these advances, the etiology of neonatal diabetes is still not known in at least 30% of patients with PNDM, suggesting other genetic causes are still to be found.

[1.4-1] Regulation of Tissue-Specific Thyroid Hormone Action
AC Bianco. University of Miami Miller School of Medicine, Miami, FL.
In all vertebrates, the iodothyronine deiodinases are critical for the biological effects mediated by thyroid hormone as they initiate or terminate thyroid hormone action. The activating deiodinase (D2) and the inactivating deiodinase (D3) control thyroid hormone action at the cellular level, increasing or decreasing thyroid hormone signaling in a tissue- and temporal-specific fashion, independently of changes in thyroid hormone
serum concentrations. From a broad perspective, deiodination can be seen as an example of a paradigm in which hormones are activated or inactivated in a controlled fashion in specific extraglandular tissues, in a role analogous to that of 5α-reductase and P450 aromatase in sex steroid metabolism and of 11β-hydroxysteroid dehydrogenase in glucocorticoid metabolism. These deiodinase-based mechanisms are indeed physiologically relevant during development and after birth, throughout health and disease. In the embryo, there are rapid reciprocal changes in D2 and D3 expression, modulating thyroid hormone signaling during critical tissue-specific developmental check-points such as in the brown adipose tissue. This also takes place in the hypothalamus and pituitary gland, ultimately regulating TSH secretion. After birth, thyroid hormone activation through deiodination (via D2) plays a role in energy homeostasis, accelerating T3 production to increase energy expenditure during cold exposure or feeding a hypercaloric diet. On the other hand, in tissue injury and disease states there is induction of the inactivation pathway (via D3) that can be tissue-specific or widespread, in which case it underlies the development of the “euthyroid sick syndrome”. In all of these settings, the expression of the deiodinases is modulated transcriptionally and post-transcriptionally by a wide variety of endogenous signaling molecules such as sonic hedgehog, NF-kB, growth factors, bile acids, HIF-1α, as well as a growing number of xenobiotic substances and chemical chaperones. Thus, deiodinases seem to play a much broader role than once thought, with great ramifications for the control of thyroid hormone signaling during vertebrate development, as well as injury response, tissue repair, hypothalamic function, and energy homeostasis in adults. The therapeutic potential is obvious: if the D2 and D3 pathways can be harnessed pharmacologically, the resulting control of thyroid hormone action on a tissue-specific fashion may prove to be particularly useful.

[L4-2] Bone Morphogenetic Proteins in the Musculoskeletal System
V Rosen. Harvard School of Dental Medicine, Boston, MA.
BMPs are locally acting signaling molecules that were identified over 20 years ago based on their ability to initiate ectopic bone formation in adult animals. Subsequent gain and loss of function studies in mice demonstrated that BMPs play an essential role in the development of nearly all vertebrate organs, including those that make up the musculoskeletal system. Musculoskeletal cells remain BMP targets throughout life when BMP signaling affects both the growth and repair potentials of bone, tendon, ligament, muscle, and articular cartilage. In these tissues, BMP signaling is part of a complex signaling cascade that orchestrates the interactions of Wnt, PTH, and IGF signaling to maintain musculoskeletal tissue homeostasis.

[L5-1] Edwin B Astwood Award Lecture: Protein Folding Homeostasis & Metabolic Regulation
D Ron. University of Cambridge, Cambridge, UK.
A significant portion of intercellular communication depends on protein secretion and interpretation of extracellular signals by transmembrane receptors. Proteins of both classes fold into their functional state in the lumen of the endoplasmic reticulum (ER), a process that is assisted by a dedicated machinery of ER-associated chaperones, enzymes and other components. The magnitude of this apparatus is matched to the load of unfolded proteins confronting the ER by a cell autonomous signalling pathway referred to as the Unfolded Protein Response. The biological significance of the unfolded protein stress (ER stress) that the UPR counteracts is attested to by the deleterious consequences of mutations in signalling components of the UPR or in elements of the ER protein folding machinery. The study of extreme cases of failure of protein folding homeostasis in the secretory pathway has revealed its impact on endocrine signalling, with the endocrine pancreas manifesting special susceptibility. Of potentially greater clinical significance are hints that long term exposure to low levels of ER stress may contribute to attrition in secretory function, which may play a role in common forms of diabetes mellitus. Intriguingly, factors that influence protein folding homeostasis in the ER also have an evolutionarily-conserved dialectic relationship with intermediary metabolism and with
its hormonal control. This is reflected in the influence of nutrient availability on protein folding in the ER and in an overlap between the readouts of the mammalian UPR and that of other pathways detect nutrient sufficiency. In this talk I will attempt to summarize the aforementioned relationships and discuss their physiological and pathophysiological significance.

[5.5-2] Glucocorticoids in Fetal Programming
_JR Seckl. University of Edinburgh, Edinburgh, UK._

Epidemiological evidence suggests that an adverse fetal environment permanently programmes physiology leading to increased risks of cardiometabolic, neuroendocrine and psychiatric disorders in adulthood. We originally hypothesised that fetal glucocorticoid overexposure might explain this link. In rodents, prenatal stress, glucocorticoid exposure or inhibition/knockout of 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), the feto-placental 'barrier' to maternal glucocorticoids, reduces birth weight and causes permanent hypertension, hyperglycaemia, increased hypothalamic-pituitary-adrenal (HPA) axis activity and anxiety-related behaviours in adult offspring. The phenotype persists into a second generation and transmits via male and female lines. This implies epigenetic mediation, a mechanism emerging for HPA axis programming. This also appears of potential clinical relevance. Thus, in non-human primates, exposure to glucocorticoids in the second half of gestation programmes cardiometabolic, HPA and behavioural parameters in offspring. In humans, placental 11β-HSD2 activity correlates directly with birth weight and inversely with infant blood pressure. Moreover, low birth weight babies have higher plasma cortisol levels throughout adult life, indicating HPA programming. Indeed, maternal glucocorticoid therapy or ingestion of liquorice (which inhibits 11β-HSD) alters offspring cognition, behaviour and HPA function. Stress has similar effects since pregnant women exposed to the 9.11.2001 atrocity and who developed PTSD 'transmit' neuroendocrine changes to their one-year old offspring, but confined to third trimester exposure. Furthermore, exposure to the Nazi Holocaust exerted permanent effects upon glucocorticoid levels and steroid metabolism, effects dependent upon the age at exposure. The second (un-exposed) generation also shows altered cortisol levels and metabolism. Overall, the data suggest that developmental exposure to excess glucocorticoids/stress programmes peripheral and CNS functions in adult life, predisposing to affective and other pathology, and these effects may impact on a subsequent generation.

[7.1-1] Roy O Greep Award Lecture: Epigenetics & Metabolism: The Circadian Link
_P Sassone-Corsi. University of California, Irvine, CA._

Extracts from a recent publication by the speaker:
_J Cell Sci 123, 3837-3848 Mammalian circadian clock and metabolism – the epigenetic link_, Marina Maria Bellet and Paolo Sassone-Corsi

Department of Pharmacology, Unite 904 Inserm, School of Medicine, University of California, Irvine, CA, USA.

Summary

Circadian rhythms regulate a wide variety of physiological and metabolic processes. The clock machinery comprises complex transcriptional–translational feedback loops that, through the action of specific transcription factors, modulate the expression of as many as 10% of cellular transcripts. This marked change in gene expression necessarily implicates a global regulation of chromatin remodeling. Indeed, various descriptive studies have indicated that histone modifications occur at promoters of clock-controlled genes (CCGs) in a circadian manner. The finding that CLOCK, a transcription factor crucial for circadian function, has intrinsic histone acetyl transferase (HAT) activity has paved the way to unraveling the molecular mechanisms that govern circadian chromatin remodeling. A search for the histone deacetylase (HDAC) that counterbalances CLOCK activity revealed that SIRT1, a nicotinamide adenine dinucleotide (NAD+)-dependent HDAC, functions in a circadian manner. Importantly, SIRT1 is a regulator of aging, inflammation and metabolism. As many transcripts that oscillate in mammalian peripheral tissues encode proteins that have central roles in metabolic processes, these
findings establish a functional and molecular link between energy balance, chromatin remodeling and circadian physiology. Here we review recent studies that support the existence of this link and discuss their implications for understanding mammalian physiology and pathology.

Conclusions. How extensive is the interplay between cellular metabolism and circadian cycles? It is currently difficult to fully address this question, but accumulating evidence shows there is a mutual relationship – the clock controls some crucial metabolic pathways and metabolism feeds back to the clock machinery (Eckel-Mahan and Sassone-Corsi, 2009). In this sense, the classic scheme of the circadian pacemaker in peripheral clocks could be conceptually modified to include the potential role of metabolic outputs, such as NAD+, in feeding back to the pacemaker and acting as adjusting signals. How complex and varied this ‘adjusting’ activity is it is not yet understood, but we predict that it could be more extensive than currently known. One additional level of complexity is tissue specificity. It is highly probable that different sets of metabolites oscillate with varied amplitude or period in different tissues. Here, it would be highly informative to compare the scenario of the circadian regulation in different peripheral tissues with the clock function in the SCN. Further circadian metabolomic studies should at the very least provide a more complete picture of which metabolites oscillate and when. However, a crucial challenge will be to identify and decipher the specific molecular pathways that determine each particular oscillation. We anticipate that these studies will provide valuable leads to develop successful therapeutic intervention of metabolic disorders.


RB Simerly. University of Southern California, Los Angeles, CA.

Extracts from a recent publication by the speaker:


Abstract. For more than a century, clinical investigators have focused on early life as a source of adult psychopathology. Early theories about psychic conflict and toxic parenting have been replaced by more recent formulations of complex interactions of genes and environment. Although the hypothesized mechanisms have evolved, a central notion remains: early life is a period of unique sensitivity during which experience confers enduring effects. The mechanisms for these effects remain almost as much a mystery today as they were a century ago. Recent studies suggest that maternal diet can program offspring growth and metabolic pathways, altering lifelong susceptibility to diabetes and obesity. If maternal psychosocial experience has similar programming effects on the developing offspring, one might expect a comparable contribution to neurodevelopmental disorders, including affective disorders, schizophrenia, autism, and eating disorders. Due to their early onset, prevalence, and chronicity, some of these disorders, such as depression and schizophrenia, are among the highest causes of disability worldwide according to the World Health Organization 2002 report. Consideration of the early life programming and transcriptional regulation in adult exposures supports a critical need to understand epigenetic mechanisms as a critical determinant in disease predisposition. Incorporating the latest insight gained from clinical and epidemiological studies with potential epigenetic mechanisms from basic research, the following review summarizes findings from a workshop on Early Life Programming and Neurodevelopmental Disorders held at the University of Pennsylvania in 2009.

Introduction. Historically, the term epigenetics has referred to heritable traits that are not mediated by changes in DNA sequence. More recently, epigenetics has been used more broadly to refer to any change in gene function not associated with sequence variation and has been embraced by the neuroscience community as a means by which we can integrate a role for the environment to influence or “program” gene expression
or patterns that may or may not be heritable. Epigenetic mechanisms typically involve DNA methylation, histone acetylation, and noncoding RNAs, including microRNAs. Increasing evidence shows that numerous types of chromatin modifications, referred to as chromatin remodeling, are widespread in the brain and undergo dynamic regulation in both the developing and adult nervous system. Incorporating the latest insight gained from clinical and epidemiological studies with potential epigenetic mechanisms from basic research, the following report summarizes findings discussed at a recent conference on Early Life Programming and Neurodevelopmental Disorders held at the University of Pennsylvania. The conference was thematically based on identifying common mechanisms that may underlie neurodevelopmental disease predisposition and included prenatal, postnatal, and early developmental determinants such as stress experience and maternal diet, behavior, and infection. The goal of the conference and this report is to disseminate the most recent findings across epidemiological, clinical, and basic science in early life programming to inform new directions and needs in the field. These findings are discussed below subdivided into areas of disease focus.

Main sub-titles:
- Fetal Antecedents and Programming in Neurodevelopmental Disorders: Schizophrenia, Affective Disorders
- Animal Models
- Programming Effects of Maternal Diet and Early Nutrition
- What Does Sex Have to Do with It?
- Epigenetic Mechanisms for Stable Behavioral Modification

Conclusions. At the crossroads of the developing brain and the perturbations poised to promote any deviation from this norm may lay the programming events contributing to disease susceptibility or resistance. Studies aimed at this level afford us a great opportunity to define disease mechanisms and identify novel targets in therapy and prevention. The interaction of the clinical, epidemiological, and basic science communities is essential in evaluation of study outcomes and in defining future directions and needs. New mechanisms and models developed at the bench will inform clinicians as to potential markers and targets to be examined at the bedside, with novel clinical observations then characterized for underlying mechanisms in animal models. Highly innovative tools and techniques are continuously being developed with greater and greater depths of analyses that will, no doubt, in the near future identify an array of novel genes and epigenetic mechanisms involved in the development of neuropsychiatric diseases. It is crucial that as this fast-moving field progresses, conversations at all levels and across disease areas continue and forums for such dialogue continue to be encouraged and supported.

[L8-1] microRNAs & Their Targets in Pregnancy & Labor
*CR Mendelson. University of Texas Southwestern Medical Center, Dallas, TX.*

Extracts from a recent publication by the speaker:
*Proc Natl Acad Sci U S A.* 2010 Nov 30;107(48):20828-33. miR-200 family and targets, ZEB1 and ZEB2, modulate uterine quiescence and contractility during pregnancy and labor. Renthal NE, Chen CC, Williams KC, Gerard RD, Prange-Kiel J, Mendelson CR. Department of Biochemistry, North Texas March of Dimes Birth Defects Center, University of Texas Southwestern Medical Center, Dallas, TX, USA.

Abstract. Throughout most of pregnancy, uterine quiescence is maintained by increased progesterone receptor (PR) transcriptional activity, whereas spontaneous labor is initiated/facilitated by a concerted series of biochemical events that activate inflammatory pathways and have a negative impact on PR function. In this study, we uncovered a previously undescribed regulatory pathway whereby micro-RNAs (miRNAs) serve as hormonally modulated and conserved mediators of contraction-associated genes in the pregnant uterus in the mouse and human. Using miRNA and gene expression microarray analyses of uterine tissues, we identified a conserved family of miRNAs, the miR-200 family, that is highly induced at term in both mice and humans as well as two coordinately down-regulated targets, zinc finger E-box binding
homeobox proteins ZEB1 and ZEB2, which act as transcriptional repressors. We also observed up-regulation of the miR-200 family and down-regulation of ZEB1 and ZEB2 in two different mouse models of preterm labor. We further demonstrated that ZEB1 is directly up-regulated by the action of progesterone (P(4))/PR at the ZEB1 promoter. Excitingly, we observed that ZEB1 and ZEB2 inhibit expression of the contraction-associated genes, oxytocin receptor and connexin-43, and block oxytocin-induced contractility in human myometrial cells. Together, these findings implicate the miR-200 family and their targets, ZEB1 and ZEB2, as unique P(4)/PR-mediated regulators of uterine quiescence and contractility during pregnancy and labor and shed light on the molecular mechanisms involved in preterm birth.

**Introduction.** Although premature labor is the leading cause of neonatal morbidity and mortality in developed countries, the signalling mechanisms that maintain uterine quiescence during pregnancy and promote increased uterine contractility leading to labor at term and preterm remain incompletely defined. In mammalian pregnancy, uterine quiescence is maintained by elevated circulating progesterone (P4) acting via the progesterone receptor (PR). Conversely, parturition is associated with a decline in maternal circulating P4 and/or a decrease in the function of the PR, termed “functional P4 withdrawal,” and an increased inflammatory response within the uterus and cervix. Studies from a number of laboratories, including our own, suggest that P4 and PR maintain uterine quiescence until term by inhibiting expression of contraction-associated genes [e.g., connexin-43 (CXN-43), oxytocin receptor (OXTR), cyclooxygenase 2 (COX-2)] in the myometrium, in part, via anti-inflammatory actions. For example, P4 and PR inhibit activation of COX-2 expression in myometrial cells through direct interaction of PR with NF-κB p65 and by P4-induced expression of the NF-κB inhibitor, IκB-α. Recently, it has been shown that micro-RNAs (miRNAs) play especially powerful roles in vascular smooth muscle cells and in female reproduction, wherein they have been implicated in proliferation, differentiation, and hormone responsiveness. The identification of miRNAs as hormonally regulated modulators of gene expression prompted us to investigate their roles in P4 and PR regulation of contraction-associated genes during pregnancy and labor. In the present study, we show that members of the miR-200 family in both the mouse and human uterus are significantly induced during late gestation, repress the zinc finger E-box binding homeobox proteins ZEB1 and ZEB2, and mediate myometrial contractility. Through overexpression experiments, we show that miR-200s repress endogenous ZEB1 and ZEB2 expression in human myometrial cells. By overexpressing ZEB1 and ZEB2 in these cells, we establish that these transcription factors markedly suppress expression of the contraction-associated genes OXTR and CXN-43. Together, our findings implicate the miR-200 family and their targets, ZEB1 and ZEB2, as unique P4- and PR-regulated modulators of uterine quiescence and contractility during pregnancy and in term and preterm labor.

**Final Comment.** Collectively, our findings suggest that ZEB1 is a key PR target gene in the myometrium that inhibits expression of contraction associated genes and the miR-200 family throughout most of pregnancy. Near term, signals from the fetus and mother cause an increased inflammatory response, leading to a decline in local P4 and/or PR function and the activation of contraction-associated genes. The decline in PR function near term causes a down-regulation of ZEB1 gene expression, which, in turn, results in derepression and up-regulation of miR-200b/429 expression. The resulting elevated miRNAs can then feed back and repress both ZEB1 and ZEB2. This negative feedback loop, which is supported by the present findings in the pregnant mouse and human myometrium and in cultured human myometrial cells as well as by published reports by others, results in further induction of contraction-associated gene expression and labor. Taken together, our findings implicate a previously undiscovered pathway in the regulation of uterine contractility during pregnancy and parturition that is conserved from mice to humans and may ultimately open avenues for development of effective therapeutics for prevention of preterm labor.

**[L8-2] Extra-Nuclear Steroid Receptor Functions**

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Classical sex steroid receptors traffic to the plasma membrane after palmitoylation by recently identified acyltransferase and facilitator proteins. Membrane-localized receptors engage small G proteins to cause calcium flux and cyclic nucleotide generation in seconds, and kinase cascade activation in several minutes. Estrogen (ER), progesterone, and androgen receptors traffic to the plasma membrane of hormone responsive-cancer cells, rapidly signaling to proliferation and survival. Gene transcription resulting from nuclear steroid receptor action often depends upon membrane-localized receptors rapidly signaling through ERK and other kinase pathways to cause epigenetic modifications. Mitochondrial ERβ prevents apoptosis in lung and breast cancer, also mediating the response or resistance to tamoxifen in breast cancer cells. Extra-nuclear steroid receptors play important roles in cardiovascular, central nervous, and bone systems. ER functions include preventing cardiac hypertrophy, fibrosis, and acute vascular injury, stabilizing osteoblasts and preventing osteoclast formation, and consolidating memory while stimulating dendritic spine formation in the brain. Using a mouse created to express only membrane-localized, E domain of ERα while lacking nuclear ERα, estrogen rapid signaling impacts cell metabolism in the absence of the nuclear steroid receptor.

SYMPOSIA


[S17-1] Genetic Variation & Timing of Puberty

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Age at menarche varies widely between girls, is highly heritable, but also highly dependent on nutritional status. While rare deleterious mutations are increasingly reported in genes of the hypothalamic-gonadal-sex hormone pathway, those are too infrequent to explain the normal variation in pubertal timing. Recent genome-wide association (GWA) studies that genotype hundreds of thousands of single nucleotide polymorphisms (SNPs) located across the entire genome have identified many common genetic variants that are robustly associated with menarche timing.

In 2009 four separate GWA studies identified common variants in the gene LIN28B on chromosome 6 as having 'genome-wide significant' (P<5×10⁻⁸) associations with menarche timing. Effect sizes on menarche timing were ~6 weeks per allele, consistent associations were also seen with other puberty traits in both girls and boys, and a mouse model has since confirmed its biological role. LIN28B is homologous to the ancestral heterochromic gene lin-28 in Caenorhabditis elegans and these findings point to a fundamental microRNA processing system that controls the tempo of both cellular and somatic development.

In 2010 the large-scale “REPROGEN” collaboration, comprising the four original menarche GWA studies and also several others, identified a total of 32 loci for menarche timing. Notably, several menarche loci are also loci for adult body mass index, and others are implicated in energy homeostasis (BSX, CRTC1 and MCHR2) and hormone regulation (INHBA, PCSK2 and RXRG). Pathway analyses identified coenzyme A and fatty acid biosynthesis as biological processes related to menarche timing.

In summary, GWA studies for age at menarche demonstrate how the genome-wide approach can help identify new mechanisms in pubertal timing and in particular how nutritional status might signal on the hypothalamic-gonadal pathway.


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Puberty is a tightly regulated process by which an individual attains the ability to reproduce. An intricate network of central and peripheral factors, including metabolic cues, has been shown to play a role in this process; however, the mechanism triggering puberty onset remains largely unknown. Recent evidence suggests that the neuropeptides kisspeptin (encoded by Kiss1) and neurokinin B (NKB; encoded by
TAC3 in humans and Tac2 in rodents) are essential gatekeepers of puberty onset. Studies in humans and rodents have shown that loss-of-function mutations in either KISS1 or TAC3, or their receptors, KISS1R and TACR3, respectively, lead to the absence of sexual maturation and infertility. As a result, reproductive neuroendocrinologists have begun to study the neurons that express these genes, attempting to discover how they interact and how they are regulated. We and others have shown that kisspeptin, NKB and dynorphin A (Pdyn) are co-expressed in neurons of the arcuate nucleus, so called KNDy neurons. Importantly, these neurons also co-express the NKB receptor, Tacr3. Kisspeptin stimulates the release of gonadotropin-releasing hormone (GnRH) by acting through Kiss1R expressed in GnRH neurons. Furthermore, NKB has been shown to stimulate LH release, presumably by acting directly on KNDy neurons to induce kisspeptin-mediated GnRH secretion. Additionally, we have shown that Tac2 and Tacr3 mRNA levels are increased at the time of puberty onset in the rat, and are inhibited by caloric restriction. Based on these observations, along with compelling evidence suggesting that GnRH pulses are associated with kisspeptin pulses, we have proposed a model whereby KNDy neurons drive pulsatile GnRH secretion. According to this model, the stimulatory action of NKB, coordinated with the delayed inhibitory action of dynorphin, generates the pulsatile release of kisspeptin from KNDy neurons by a mechanism that is dependent on sex steroid levels. Understanding how this pulse generator is activated during puberty and remains functional in adulthood is the goal of recent research in reproductive neuroendocrinology.

[S17-3] Epigenetic Mechanisms Involved in the Control of the Onset of Puberty
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The onset of puberty requires many genes, which play different roles along a developmental cascade leading to the pubertal increase in GnRH release. It is unclear, however, how inherited, permanent changes in DNA sequence can dynamically coordinate the expression of gene sets controlling the pubertal process. Epigenetics is able to do this. Here we propose that an epigenetic mechanism of transcriptional repression plays a significant role in timing the initiation of mammalian puberty. Using Illumina and Affymetrix arrays, Nimblegen genome–wide DNA methylation analyses, and quantitative PCR, we identified genes of the Polycomb group (PcG) of transcriptional silencers as major contributors to this repressive mechanism. Expression of two key PcG genes, Cbx7 and Eed, decreases in the hypothalamus preceding the onset of puberty, and this decrease is accompanied by increased methylation of their promoters. In vivo inhibition of de novo DNA methylation with 5'-azacytidine (5-Aza), restored PcG expression, and resulted in pubertal failure (as assessed by the lack of ovulation). The treatment did not affect the ovarian estradiol response to gonadotropins, but it prevented the LH response to ovariectomy, suggesting that puberty is delayed due to a central, instead of an ovarian defect. Both the LH response to GnRH and the GnRH response to kisspeptin were normal, indicating that the defect is upstream of the GnRH network. ChIP assays showed that PcG proteins interact with the Kiss1 promoter, and that puberty is accompanied by Kiss1 promoter demethylation, association of activating histones (H3K9,14ac and H3K4me3), and loss of H3K27me3, a repressive histone form catalyzed by PcG proteins. Preventing the peripubertal decrease in hypothalamic Cbx7/Eed by microinjecting Cbx7 or Eed-producing lentiviruses into the arcuate nucleus of prepubertal rats resulted in association of these proteins to the Kiss1 promoter, a striking delay in puberty, and disruption of estrous cyclicity. Some animals failed to become pregnant when exposed to a fertile male. These results suggest that an epigenetic mechanism of transcriptional repression plays a significant role in timing the initiation of mammalian puberty, and that the PcG group of transcriptional silencers is a major contributor to this repressive mechanism. PcG proteins appear to target downstream genes involved in the stimulatory control of GnRH secretion at puberty, such as the Kiss1 gene.

SYMPOSIUM: TRANSLATIONAL - Insulin-Like Growth Factor System & Growth: Basic Mechanisms
accretion during prepubertal growth is predominantly mediated by TH axis in mice. Based on these and other findings, we concluded that IGF-I gene and thereby regulates the promoter activity of IGF-I gene. Based on these and other findings, we conclude that IGF-I expression and bone accretion during prepubertal growth is predominantly mediated by TH axis in mice.

[S26-1] IGF-I Role in Skeletal Acquisition: Lessons from Mouse Models
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The best protection against future osteoporosis and bone fragility is to “grow the right skeleton”. The GH/IGF-1 axis plays a major role in determining bone size and mass acquired during growth in humans and animals. Nonetheless, over the last decade, much of the research on the GH/IGF-1 axis in bone has focused on its potential role in post-menopausal and age-related osteoporoses. The overwhelming consensus of these studies is that decreases in IGF-1 and GH with menopause and aging coincide and do not appear to be the major cause driving osteoporosis. Studies from our as well as other laboratories clearly show that modulation of the GH/IGF-1 axis during growth has major effects on skeletal integrity that are carried on into adulthood and aging. As GH/IGF-1 axis has two modes of actions i.e. the endocrine (serum) and autocrine/paracrine (tissue), studies in the last decade addressed how selective changes in each of the modes affect skeletal integrity. We found that alterations in serum IGF-1 levels have minimal effects on bone length, but appear to control radial/transversal bone growth. Likewise, mouse models with reduced serum IGF-1 levels, such as the liver IGF-1 deficient (LID) or the acid labile subunit KO mice (ALSKO), develop slender bones, owing to inhibition of periosteal osteogenesis, which can be reversed by GH treatment. In contrast, mice overexpressing hepatic IGF-1 transgene (HIT), with elevations in serum IGF-1 show more robust bones, independent of tissue IGF-1 (KO-HIT model). Interestingly, however, knocking out the GHR impairs both longitudinal and transverse bone growth, such that periosteal apposition cannot be rescued by elevations in serum IGF-1. This may suggest that GH-dependent tissue factors control transversal bone growth. Since transversal bone growth is one of the most critical determinants of bone strength throughout life, future studies should uncover how intrinsic tissue (bone/muscle) GH/IGF-1 signaling pathways control transversal periosteal bone growth.

[S26-2] Mechanisms Regulating Prepubertal Bone Growth
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Low peak bone mass is an important risk factor for osteoporosis. Therefore, studies to understand the mechanisms regulating bone accretion during postnatal growth periods are of considerable importance in the prevention and treatment of osteoporosis. With regard to potential signaling molecules which contribute to skeletal growth, the findings from a variety of transgenic mouse models and clinical studies of mutations in genes that regulate IGF-I action have illustrated a key role for IGF-I in the regulation of peak bone mass. In terms of mechanisms for IGF-I regulation, there is now irrefutable evidence for the involvement of GH axis in the regulation of IGF-I action during pubertal growth period. Based on the findings that the magnitude of deficit in bone mineral density (BMD) and bone size is much greater in IGF-I knockout mice compared to GH deficient mice at the end of prepubertal growth period, we predicted that IGF-I regulation of bone accretion during prepubertal growth period is mediated by a GH-independent mechanism. In our efforts to identify the key molecules that regulate IGF-I expression during the prepubertal growth period, we focused on thyroid hormone (TH) axis because the rapid increase in serum IGF-I levels during pubertal growth period were preceded by increased serum T3 and correlated highly with serum T3. The cause and effect relationship between changes in serum T3 and IGF-I levels was demonstrated by using two mutant mouse models, Tshr<sup>h/h</sup> and Duox2<sup>−/−</sup>, that exhibit TH deficiency. Furthermore, treatment of Tshr<sup>h/h</sup> mice during prepubertal growth period (day 5-14) with daily administration of replacement doses of T3/T4 rescued deficits in serum IGF-I, skeletal IGF-I and femur BMD. Accordingly, T3 treatment increased IGF-I expression at both mRNA and protein levels in a dose and time-dependent manner in osteoblasts. Studies on the molecular pathway for TH regulation of IGF-I expression in osteoblasts revealed that ligand bound TH receptor α1 binds to TH response element in the first intron of IGF-I gene and thereby regulates the promoter activity of IGF-I gene. Based on these and other findings, we conclude that IGF-I expression and bone accretion during prepubertal growth is predominantly mediated by TH axis in mice.
IGF Deficiency (IGFD) can result from either growth hormone (GH) deficiency or GH insensitivity. As experience with clinical conditions associated with IGFD has increased, it has become apparent that IGFD in the presence of normal GH secretion (primary IGFD) constitutes a spectrum of clinical conditions, with a range of phenotypes, biochemical characteristics and molecular defects. Over the last five years, we have performed biochemical and/or molecular analyses on ~500 patients with short stature, of whom ~200 have had heights < -3 SD. Of these, ~50 have had identifiable molecular defects resulting in either primary IGFD or IGF resistance. The molecular basis for an IGFD continuum is predicated on the following observations: 1) defects of a variety of genes may result in primary IGFD; 2) different mutations of the same gene may result in a range of phenotypes: 3) mutations can result in immunodetectable, but bioinactive proteins; 4) dominant negative mutations in classic autosomal recessive disorders may lead to a mild phenotype; 5) heterozygosity for some autosomal recessive disorders may result in an attenuated phenotype; 6) even with identical mutations of the same gene, a range of phenotypes may be observed, presumably reflecting the effects of interacting genes and polymorphisms. In an effort to catalog these defects, a website has been established for genetic defects of the GH-IGF axis:
http://growthgeneticsconsortium.org

SYMPOSIUM: TRANSLATIONAL - Humanin & the Emerging Field of Mitochondrial Peptides –

[S36-2] Humanin & Testes Biology
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Mitochondria are involved in energy metabolism and apoptosis, and are central to the pathogenesis of multiple diseases, including diabetes, cancer, neurodegeneration and aging. Mitochondria contain nearly a thousand proteins of nuclear origin, but the mitochondrial-chromosome only encodes 13 proteins. In 2001, humanin; a novel 24-amino-acid peptide proposed to be encoded from the 16S ribosomal RNA region of the mtDNA, was described to be a potent neurosurvival factor. Soon afterwards humanin was shown to bind and antagonize Bax and IGFBP-3 (1). Humanin has been shown to be cytoprotective in vitro and in vivo in models of stroke, ALS, and Alzheimer's and has metaboloprotective activities against both type-1 & type-2 diabetes (2, 3) and atherosclerosis (4). We recently developed a humanin ELISA assay and demonstrated that humanin declines with aging and its levels are altered in states of insulin resistance, diabetes, endothelial dysfunction and neurodegeneration. We recently identified an additional six peptides encoded from ORFs within the 16S rRNA, which we named SHLPs (small humanin-like peptides). Analysis of their expression reveals that they are transcribed in the mitochondria from mtDNA, are detectable in plasma, and exhibit a tissue-specific distribution. SHLPs 1-5 act as potent bioactive molecules acting to induce cell survival and ROS inhibition (like humanin, via activation of Erk and Stat3 phosphorylation) but with different temporal profiles, suggesting that these peptides may act in concert. SHLP6 has opposing actions, potently inducing apoptosis. These observations reveal that the mitochondria possess previously unappreciated roles in the regulation of metabolism and apoptosis that occur via the synthesis of mitochondrial-derived peptides (MDPs). We propose that the mitochondrial peptidome could explain important new aspects of mitochondrial biology and dysfunction with relevance to human biology and disease and that the novel MDPs we describe here may represent retrograde communication signals from the mitochondria.
Humanin (HN) is a 24 amino-acid pro-survival peptide that is expressed in immature and mature rodent testes as well as in human spermatocyte and spermatids. We have shown that intratesticular HN administration reduces germ cell apoptosis induced by gonadotropin releasing hormone antagonist (GnRH-A) in rats, suggesting the possibility that HN restrains GnRH-A's pro-apoptotic action (1). Furthermore, HN reduces spontaneous germ cell apoptosis in serum-free ex-vivo cultures of rat seminiferous tubules. IGFBP-3 is a binding partner of HN and induces apoptosis of germ cells in the testis. HN mitigates pro-apoptotic actions of IGFBP-3 on male germ cells in the ex-vivo seminiferous tubules. Another intracellular binding partner of HN is BAX. In vitro, HN binds to BAX and prevents its translocation into the mitochondria to induce the caspase cascade and apoptosis. We recently found that HN also binds to the pro-apoptotic molecule BAX in the cytoplasm of testicular cell homogenates, suggesting HN may not only negatively regulate IGFBP-3, but also BAX (2). As precedent for this, we previously demonstrated that the balance between BAX and BCL-2, an anti-apoptotic protein, is critical in dictating the frequency of germ cell apoptosis. We have also shown that BAX partners with IGFBP-3 in testis mitochondria in vivo and in vitro, suggesting these two pro-apoptotic molecules may function together in germ cells. Together, our published and preliminary findings suggest that HN prevents cell death in the testis by harnessing the actions of IGFBP-3 and BAX. We are studying whether HN functions by activating both membrane receptor-dependent and -independent pro-survival mechanisms. We propose the HN is a key regulator of testicular germ cell apoptosis. We speculate that perturbation of HN action has conserved effects on germ cell apoptosis from rodents to humans and that future studies may show that HN is a potential therapeutic target for development for male infertility, male contraception and germ cell cancer.

[S36-3] Metabolic Effects of Humanin
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Humanin (HN), a novel mitochondria-associated peptide was originally identified in surviving neurons of a patient with Alzheimer's disease. Since its identification, HN has been shown to have a broad cyto-protective profile and protect against AD related neurotoxicity in neuronal and smooth muscle vascular cells, stroke, and to attenuate memory loss in AD and scopolamine-induced models. Consistent with its cyto-protective effects, we showed that HNG is cardio-protective in the setting of myocardial ischemia reperfusion. Protection against neuronal cell death by HN results from its interaction with cytokine receptor complex, CNTFR-a/WSX-1/gp130. HN analogs created by molecular manipulations of HN such as HNG (S14G-HN) are more potent than native HN in its biological actions. We demonstrated recently that Humanin, when infused intra-cerebroventricularly, significantly improves hepatic and peripheral insulin action. The central effects of HN on insulin action are associated with activation of hypothalamic STAT-3 signaling; effects that are negated by co-inhibition of hypothalamic STAT-3. Furthermore, endogenous IGFBP-3 in the hypothalamus tempers the effects of HN on glucose metabolism. This is demonstrated by the significantly higher insulin-sensitizing effects of HN analogues that do not bind IGFBP-3. Peripheral infusions of novel HN derivatives reproduce the insulin-sensitizing effects of central HN during insulin clamp. A single injection of HNGF6A significantly decreased blood glucose levels in Zucker diabetic fatty rats. Humanin analogs also increase insulin secretion both in vivo and in vitro. We conclude that humanin has potent effects on glucose homeostasis and may represent a novel link between diabetes and neuron-degeneration.

SYMPOSIUM: TRANSLATIONAL - Adrenal Disorders in Children & Adolescents

[S54-1] Clinical Effects of Cushing Syndrome in Children
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Cushing's syndrome is rare in the pediatric age group, yet presents unique diagnostic and management challenges. This clinical session will present an overview of the work-
up and management of pediatric Cushing's syndrome, providing a brief overview of the field and highlighting recent progress. The classification and epidemiology of Cushing's syndrome in children will be discussed, along with diagnostic guidelines (1-3). Common clinical features in the presentation of Cushing's syndrome in children will be outlined. An algorithm to guide the clinician investigating Cushing's syndrome will be examined. An up-to-date review of treatment strategies of pediatric Cushing's syndrome will be presented. Particular issues related to post-treatment management in pediatric patients will focus on growth, bone health, metabolic syndrome, cardiovascular health, and puberty as they affect the developing child with Cushing's syndrome (4-6). Recommendations for the care of patients during the post-surgical period with regard to recovery of the HPA axis and tapering glucocorticoid therapy will be addressed. Finally, recent advances in the field will be introduced.

(3) Savage, M.O., et al., Curr Opin Endocrinol Diabetes Obes 2008; 15:346-51

[SS54-2] Novel Genetic Causes of Familial Glucocorticoid Deficiency
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Familial Glucocorticoid Deficiency (FGD) is a rare autosomal recessive disorder characterized by resistance to the action of ACTH and consequently glucocorticoid deficiency with preserved mineralocorticoid and gonadal function. We identified mutations in the ACTH receptor (melanocortin 2 receptor; MC2R) in 1993, although these only explained around 25% of cases. More recently a genetic approach identified mutations in a novel gene which we named melanocortin 2 receptor accessory protein (MRAP). The product of this gene is a small protein essential for trafficking of the MC2R to the cell membrane and for binding of ACTH. A similar clinical phenotype may also be produced by less severe mutations in STAR. Nevertheless approximately 50% of all cases of FGD have no genetic explanation. Use of homozygosity mapping, targeted exon capture and high throughput sequencing has recently identified two new FGD genes. These are (1) a gene essential for DNA replication, which is mutated in a phenotypic variant of FGD found exclusively in the Irish traveler population, and (2) a gene that is essential for maintenance of the mitochondrial redox state. In view of the function of this latter gene other components of the mitochondrial redox pathway were screened and an inactivating mutation in a further gene in this class was identified in FGD. In summary, new technological approaches have identified three new genes associated with FGD which reveal a range of mechanisms leading to failure of the zona fasciculata cell and development of ACTH insensitivity.

[SS54-3] Why Pediatric Adrenocortical Tumors Differ from Adult Tumors
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Adrenocortical carcinomas account for only 0.05-0.2% of all cancers, with an estimated incidence of 0.5-2 per million per year in adults (1, 2). However, the incidence of adrenocortical tumors is remarkably high in Southern Brazil, where it is estimated to be 10-15 times greater than the worldwide incidence (2-4). Differently from adults, pediatric adrenocortical neoplasms with apparently poor prognosis based on histopathological features have often a good clinical outcome (5, 6). To date, few molecular markers are helpful to predict poor prognosis in children with adrenocortical carcinomas (1, 7-10). In the last decade, considerable advances toward understanding the molecular mechanisms of adrenocortical tumorigenesis have mainly been made in adrenocortical tumors of adults from distinct ethnicities (8, 11-15). Some of these studies have recently inspired the molecular analysis of adrenocortical tumors of children and adolescents, most of them from Brazilian origin (7, 16-20). A high incidence of a P53 germline mutation (p.R337H) was demonstrated in 35 of 36 children with adrenocortical tumors originated from Southern Brazil (16). This mutation was
located outside the highly conserved DNA binding domain, resulting in the substitution of arginine for histidine at codon 337 of the tetramerization domain of P53 protein. This missense mutation was also present in 76% of children with benign and malignant sporadic adrenocortical tumors in another Brazilian series from our Institution (17). Additionaly, the p.R337H mutation was identified in 14% of adult patients with adrenocortical tumors (17). We and others demonstrated that IGF-II is over-expressed in both adult and pediatric adrenocortical tumors (7, 12-14). The mitogenic effects of IGF-II are mediated by the IGF receptor type 1 (IGF-IR) (21). Interestingly, a strong increase in IGF-IR expression was found in pediatric adrenocortical carcinomas, but not in adult carcinomas (7). Furthermore, a selective IGF-IR kinase inhibitor had anti-tumor effects in adult and pediatric adrenocortical tumor cell lines (7). Recent studies revealed an important role for the steroidogenic factor-1 (SF-1) in adrenocortical tumorigenesis (19). More recently, we demonstrated a higher frequency of SF-1 over-expression and gene amplification in pediatric than in adult adrenocortical tumors (20). Therefore, we conclude that adrenocortical tumors in children and adults have different mechanisms of tumorigenesis.