S2-2

Molecular Mechanisms of Neuroendocrine Control of Reproduction

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Reproductive function is controlled by rhythms of hypothalamic secretion of GnRH into the hypophyseal portal system. These pulses of GnRH reach the anterior pituitary to activate the transcription and secretion of LH and FSH from the gonadotrope cells. Utilizing immortalized GnRH neurons and pituitary gonadotrope cells as cultured cell models, as well as genetically altered mice, we have investigated the basis of the expression of the GnRH gene, the rhythmic secretion of GnRH, differential responsiveness of the LH and FSH genes to GnRH, and the developmental specificity of expression of the LH and FSH genes.

S2-3

Homeobox genes in pituitary development and disease

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Prop1, Lhx4 and Pitx2 are among the homeobox genes that are critical for normal pituitary development and function. Lesions in PROP1 affect only the pituitary and are the most common cause of multiple pituitary hormone deficiencies in humans. LHX4 mutations affect the development of the cerebellum as well as the pituitary, and PITX2 defects cause Rieger syndrome, which includes defects of the eyes, teeth, heart, umbilicus, and pituitary. Mouse models have been invaluable in the identification and functional analysis of these critical genes. A variety of alleles can be generated that vary in severity, allowing the dosage effect of genes to be ascertained, and alleles can be produced so that the genetic deficiency is induced at a particular time of development or in a specific tissue. We have compared three mutant alleles of Prop1. Complete loss of function causes dysfunction of the pituitary adrenal axis, respiratory distress and neonatal lethality. Partial loss of function is compatible with life but causes lack of GH, TSH, PRL and reduced gonadotropins. Gain of function causes transient hypogonadism, adult onset hypothyroidism and pituitary adenoma formation. Analysis of Prop1 and Lhx4 double mutants revealed that Prop1 has critical activating and repressing roles, while the primary role of Lhx4 appears to be in supporting cell survival. A series of Pitx2 alleles revealed distinct roles for Pitx2 at different developmental times. It is required early for expansion of the pituitary primoridium and later for differentiation of the gonadotrope lineage. Its expression in adult pituitary gonadotropes and thyrotropes suggests that it plays a role in regulation of thyrotropin and gonadotropin production. Analysis of mouse models has revealed interactions between many of the critical genes and suggests a molecular basis for the complexities in human endocrine disease.

S2-4

Function and structure of folliculo-stellte cells in the anterior pituitary gland

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Folliculo-stellate cells (FS cells) in the anterior pituitary gland are star-shaped agranular cell and are known to produce many cytokines or growth factors, such as IL-6, LIF, bFGF and VEGF but they do not produce any pituitary hrmone. The functions of FS-cells are still under discussion, however, it is generally accepted that FS-cells work as sustentacular cells or scavenger cells. In order to reveal structure and function of FS cells we performed immunocytochemical and cytophysiological study using pituitary gland and some cell lines derived from FS cells. In our first study, we interested the many follicular colloids are formed in the senescent porcine pituitary gland. These colloids are structurally stable and allowed to purify and analyze their composition. The major component of the colloids is clusterin which is a highly glycosilated protein. By our immunocytochemical study, we proposed that clustrin producing endocrine cells are undergone to cell death and are phagocytosed with FS cells. The clusterin in the phagocytosed cells is accumulated in the colloids as a residual body. In addition of phagocytotic or supportive cell activity, we are interesting about differentiation potential of FS cells. Interestingly it is recently reported that FS-cells are positive for ptx-1, which is known as a panpituitary transacting factor. This suggests a close relationship between FS cells and endocrine cells, i.e., they both may differentiate from a common ancestral cell line. It is also noteworthy that FS cells are frequently found surrounding immature endocrine cells. These results support that at least some FS cells in the pituitary gland are progenitor cell of endocrine cells.

S2-5

Regulation of Synergy in the Pituitary

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Our cytochemical studies have revealed a synergy between anterior pituitary (AP) somatotropes and gonadotropes. Subsets of somatotropes co-express growth hormone (GH) and gonadotropins during key pre-ovulatory events. Because of its facilitatory role in reproduction, GH itself has been called a co-gonadotropin. The objective of our studies has been to learn more about the role and regulation of somatotropes in their support of the gonads, focusing on estrogen as a key regulator. AP cells from diestrous rats were treated for 24–48 hours with 100 pM estrogen and then exposed to Biotinylated Gonadotropin releasing hormone (Bio-GnRH) or Bio-GH releasing hormone (Bio-GHRH) (10 min). The analogs were detected cytochemically with avidin peroxidase; target cells were identified by immunolabeling for GH. Estrogen stimulated significant increases in % of AP cells with GH antigens and GHRH receptors (from 27±3% to 38±3%). It also stimulated more somatotropes to express GnRH receptors (from 34% to 79% of GH cells). To test the importance of GHRH receptor in reproduction, a potent GHRH antagonist (MZ4-169) was used. After two weeks of treatment, MZ4-169 blocked estrous cycles in females and caused infertility in males. Gonadotropes responded as if the rats had been castrated. Collectively, these studies suggest that GHRH and/or GH may be vital for fertility. Estrogen increases % of GH cells and their co-expression of GHRH and GnRH receptors. Estrogen may work synergistically with GHRH and GnRH to facilitate the co-gonadotropic functions of somatotropes. Supported by NIH R01 HD/DK 33915

S2-6

Functional morphology of normal and diseased thyroid

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Recent our studies on functional morphology of hormone production and angiogenesis in normal and neoplastic thyroid tissues using molecular techniques as well as conventional morphological approaches are presented.
Thyroid transcription factor-1 (TTF-1) is important for thyroid-specific and maximal expressions for thyroglobulin (TG), thyroperoxidase (TPO), and sodium/iodide symporter (NIS) genes. In our

immunohistochemical observations, TTF-1 was localized in the nuclei of normal follicular cells. In situ hybridization using an anti-sense probe revealed that expression levels of TTF-1 mRNA in follicular cells were not uniform among follicles. This finding suggests functional follicular heterogeneity of thyroid TTF-1 was also demonstrated in the nuclei of parafollicular (C) cells and hypothesized that TTF-1 similarly coordinates Ca2+-dependent gene expression in C cells. Most thyroid neoplasms including both follicular cell and parafollicular cell origins expressed TTF-1 in connection with their functional ability. On the other hand, angiostructure and angiogrowth can be asscociated with hormone production and tumor growth. Our investigation suggested that the fundamental vascular pattern varies by histological tumor type and appears to correlate well with the growth pattern, suggesting interdependence between parenchyma and stroma characteristics of thyroid tumors. TTF-1 could regulate the VEGF expression in the thyroid cells. Therefore, the investigation of TTF-1 provides useful information on the functional activities and/or differentiation of thyroid tumors and may lead to an increase in our understanding of the biologic nature.

S2-7

Analysis of Homogeneous Populations of Folliculo Stellate Cells by Laser Captive Microdissection and RT-PCR

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Pituitary folliculostellate (FS) cells are usually located between the secretory cells in the anterior pituitary and they produce many peptides that exert a paracrine effect on the hormone producing pituitary cells. We used a combination of immunostaining with S100 protein followed by laser capture microdissection (Immuno-LCM) to obtain purified population of rat FS cells. These cells were analyzed along with the FS cell line (TtT/GF) by RT-PCR.
Similar mRNA profiles of peptides were obtained with both FS cell populations and TGFB1 stimulated proliferation in both groups. However, TGFB1, but not PACAP, stimulated leptin expression in normal FS cells and inhibited leptin expression in the TtT/GF cells suggesting alterations in signal transduction in this cell line.

PL1

Architecture of the Postsynaptic Density

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A major frontier in structural biology is to unravel the structure of complex protein machines. An example of a molecular machine of great importance in neurobiology is the post synaptic density (PSD). The PSD, a coin-shaped structure 300 nm in diameter, includes multiple receptor systems, inherent structural elements, systems for linking the receptors, and various regulatory enzymes. Multiple approaches are used to study the structure of the PSD. PSDs isolated by cell fractionation techniques are adhered to glass, labeled with colloidal gold antibodies, freeze-dried and replicated, and viewed by electron microscopy (EM). A lattice labeling for the structural element PSD-95 is revealed on both surfaces of the PSD, but a kinase-CaMKII-found in large amounts in the PSD fraction is localized on the cytoplasmic surfaces of the PSD. The exact distributions of labels are compared and measured by performing EM tomography on the replicated samples. A second approach is to examine the PSDs in situ by EM tomography of thin sections. Neuronal cultures are prepared by high pressure freezing and freeze substitution. When the cultures are stimulated just before freezing, a striking dynamic property of the PSD is revealed. The amount of CaMKII adhering to the PSD rapidly increases during activity and reverses to rest levels within minutes. The replica approach has the advantage that components of unfixed PSDs can be localized very precisely in three dimensions by combining immuno gold with tomography, whereas the thin section tomography approach allows dynamic changes in structure to be analyzed. A future challenge is to combine immunolocalization with EM tomography of thin sections of fast frozen cultures in order to derive a detailed molecular architecture of the PSD.

MS1-1

The satellite cell—an old fashioned skeletal muscle stem cell

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We have been investigating the proliferative plasticity of skeletal muscle satellite cells. Satellite cells, situated on the myofiber surface, are the primary source of myogenic precursors in postnatal muscle. During normal growth and maintenance of myofibers the satellite cells undergo a restricted number of proliferative rounds before differentiation commitment, but can produce numerous progeny following a robust muscle injury. The mechanisms governing the magnitude of satellite cell proliferation are unclear. We have demonstrated that selective members of the fibroblast growth factor (FGF) family and hepatocyte growth factor (HGF) regulate the short-term proliferative capacity of satellite cells in isolated myofibers from growing and adult rodents. However, in this culture model the satellite cells rapidly differentiate. In contrast, progeny of satellite cells in myofibers from mice lacking MyoD remain proliferative for a long time. Hence, MyoD may act as a regulatory switch of proliferative plasticity in vivo; the repression of MyoD activity may allow satellite cells to function as self-renewed stem cells while the activation of MyoD may enforce a limited proliferation and rapid differentiation. We have additionally investigated the proliferative potential of satellite cells from very old mice (34-month-old). We concluded that satellite cells in myofibers from these old mice are unable to enter short-term proliferation without the addition of FGF2. However, satellite cells from old mice are clonable, proliferate extensively, and produce numerous differentiation-able progeny when dissociated from the muscle and cultured in a serum-rich/mitogen-rich environment. Hence, satellite cells maintain their stem-like characteristics even at the end of the life span. (Supported by NIH, USDA and BARD.)

MS1-2

The roles of stem-like myogenic cells in muscle growth and regeneration

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Skeletal muscle is composed of syncytial fibres in which the many nuclei are post-mitotic. Supply of new nuclei for growth of fibres or replacement of damaged fibres is thought to be largely provided by satellite cells that lie between the plasmalemma of the muscle fibre and the overlying basement membrane. However, these cells are not a homogeneous population and recent experiments have shown that they are the not the only source of myogenic cells, with compelling evidence of contributions from circulating stem cells allied to the haematopoietic system of the bone marrow. There is, however, little information on the extent to which these different cells contribute to myogenesis in the various contexts in which they operate. We have been studying the behaviour of satellite cell by means of various markers and by their behaviour on activation, both in vitro on isolated muscle fibres and in vivo on transplantation into degenerating muscle. By such means, we hope to progessively dissect the cellular mechanisms and the