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Meeting Report

Symposium in honor of Ralph L. Brinster celebrating 50 years of scientific breakthroughs

Philadelphia, 24-25 August 2012

ABSTRACT The Symposium speakers comprised a distinguished group of scientists from North America, Europe and Asia. The Keynote address was presented by Michael Brown, Nobel Laureate in Physiology or Medicine (1985), and a plenary lecture was presented by John Gurdon, who within the next months would receive the Nobel Prize in Physiology or Medicine (2012). The first lecture in the series was presented by Richard Palmiter, Ralph's collaborator for more than 15 years, and he provided an overview of their work together followed by Richard's subsequent exciting contributions in the area of neurobiology. Seventeen lectures were presented over the two-day Symposium by distinguished scientists, including several of Ralph's former colleagues and students. The topics covered studies on germ cells, the germline, early embryos and their differentiation, as well as exciting and unpublished studies on stem cells and reprogramming. Many of the topics presented arose from foundation experiments in which Ralph participated, and these new developments indicate the dramatic progression of studies in this area. The Symposium program and the complete lectures can be found at the following link: www.vet.upenn.edu/BrinsterSymposium

KEY WORDS: *Cell Reprogramming, Animal Transgenesis, Germ Cell Research, Pluripotent Stem Cells*

Introduction

The School of Veterinary Medicine and the University of Pennsylvania held a two-day Symposium on August 24 and 25th, 2012 as a tribute to Ralph Brinster's breakthrough scientific accomplishments and leadership during the past 50 years as a faculty member. It was organized by his colleagues, former students and postdoctoral fellows, and a number of national and international leaders - many of whom Ralph had mentored and are now leading scientists – who were invited to present their latest research findings. The symposium, attended by more than 300 scientists, took place at the School of Veterinary Medicine on the Penn campus in Philadelphia.

Ralph Brinster grew up on a farm, attended Rutgers University Agricultural College and following military service in Korea, obtained his veterinary and Ph.D. degrees at the University of Pennsylvania. Beginning in his early years on the farm, Ralph's interest in fertility, reproduction and germ cells developed continuously, and therefore it is not surprising that his more than 50 years in research have focused entirely on germline cells, the germline and the embryo. Initially, he developed culture and manipulation strategies for mouse eggs, which continue in use with little change today. He used these methods to demonstrate that teratocarcinoma stem cells will colonize the blastocyst, observations that enabled the development of the embryonic stem cell system. His efforts to understand and modify the germline resulted in his continued experiments in which he developed transgenic animals. Currently, his studies involve spermatogonial stem cells and their regulation in spermatogenesis. More details on Ralph's background are contained in the following link: www.vet.upenn.edu/BrinsterSymposium

24 August 2012

Richard Palmiter *(University of Washington)*

After the 15-year collaboration with Ralph Brinster, Richard Palmiter's lab began to use transgenic techniques and then gene-knockout technology to manipulate genes involved in neurotransmitter signaling. They spent many years making and then studying the biology of mice that were unable to make either norepinephrine or dopamine. More recently, they turned their attention to deciphering neural circuits that control appetite using cell ablation, viral transduction, and optogenetics to manipulate the activity of specific neurons and examine the effects on feeding behavior. Photoactivation of neurons in the hypothalamus that make agouti-related protein, neuropeptide Y and GABA (AgRP neurons) results in robust feeding, whereas ablation of those neurons results in starvation. The starvation phenotype is due to loss of GABA signaling to neurons in the parabrachial nucleus. One critical population of parabrachial neurons expresses calcitonin gene-related protein (CGRP). When these neurons are photoactivated, hungry mice stop eating. Activating these CGRP-expressing neurons is responsible for the anorexia that occurs with satiety and visceral malaise. Thus, activation of AgRP-expressing neurons stimulates feeding by mice that are not hungry, while activation of CGRP-expressing neurons inhibits feeding by mice that are hungry. The next problem is to determine the neural circuit(s) downstream of these neurons and where the circuits interact to control feeding behavior.

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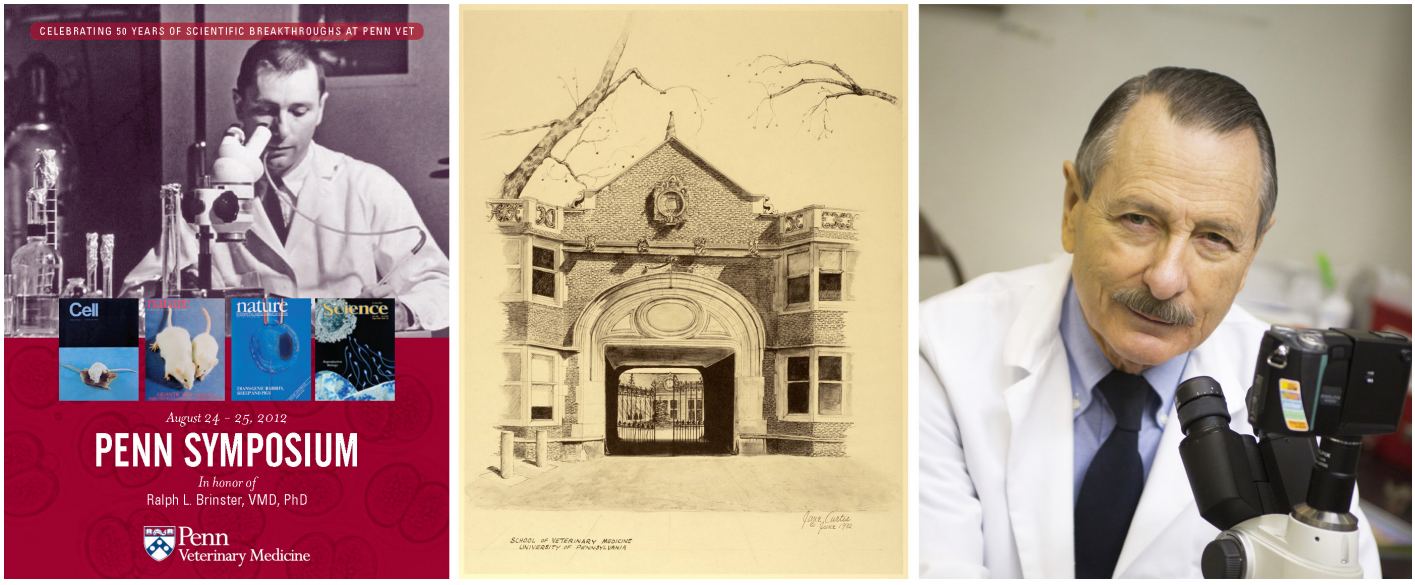


Fig. 1. A long life devoted to scientific research at the Veterinarian School. (A) *Poster of the Symposium including a picture of Ralf Brinster at the beginning of his research career,* **(B)** *Original entrance to the School of Veterinary Medicine of the University of Pennsylvania (drawing by Jane Curtis) and* **(C)** *A recent picture of Ralf Brinster.*

Richard Behringer *(University of Texas MD Anderson Cancer Center)*

Richard Behringer trained as a postdoctoral fellow with Ralph Brinster in the late 80's, focusing on transgenic approaches to study developmental biology. During this time he was also exposed to the field of reproductive biology. In his own lab he has combined both disciplines to examine the development of the mammalian reproductive organs. The Müllerian ducts are a pair of epithelial tubes with associated mesenchyme that can differentiate into female reproductive tract organs, including the oviducts, uterus, and upper portion of the vagina. They form within the mesonephroi associated with the gonads in both male and female fetuses. In males, the Müllerian ducts are eliminated by anti-Müllerian hormone (AMH) secreted by the fetal testes. In females, no AMH is produced by the fetal ovaries, creating a permissive environment for Müllerian differentiation. Descriptive and experimental embryological studies by the Behringer lab suggest that the Müllerian duct epithelial tube is generated by progenitor cells located at the tip of the growing duct. Time-lapse imaging of the forming duct using fluorescent protein transgenes in organ culture revealed unique cell behaviors of these tip cells during tubulogenesis. Mutant analysis indicates that the function of this epithelial tube is to induce the adjacent mesenchyme to differentiate into the stroma and myometrium of the uterus. New imaging paradigms for reproductive organs were also presented to reveal novel three-dimensional (3D) structures in the developing reproductive organs. Optical projection tomography (OPT) was used to generate 3D models of uterine glands and myometrium organization. These 3D structures have led to new insights into tissue differentiation, morphogenesis, and patterning. Finally, studies were presented to examine homeostasis and regeneration in the adult uterus. The mouse uterus undergoes changes in proliferation and cell death during each estrous cycle. In addition, the epithelial and stromal compartments of the uterus must be regenerated at the site of placentation after parturition. The epithelial and stromal compartments of the uteri from non-pregnant and postpartum females were examined using transgenic lineage tracing methods. The epithelial cells appear to be capable of only giving rise to epithelial cells during homeostasis and after parturition. Likewise, stromal cells appear to be capable of giving rise to stroma. However, a subset of stroma cells undergoes a mesenchymal to epithelial transition induced by parturition and stably incorporate into the luminal and glandular epithelium. This damage-induced stromal to epithelial transition may have implications for the etiology of uterine pathologies.

Rudolf Jaenisch *(Whitehead Institute, MA)*

Rudolf Jaenisch presented studies dissecting the molecular events of reprogramming during the generation of induced pluripotent stem (iPS) cells. The generation of iPS cells results in a small fraction of transfected somatic cells acquiring pluripotent states several weeks post-transfection but also many incompletely reprogrammed colonies. Molecular events occurring in these rare single cells are difficult to characterize by traditional methods of gene expression analysis. Nevertheless, through bulk analyses of cells undergoing reprogramming, a stochastic model has been proposed, in which reprogramming factors initiate a sequence of probabilistic events that lead to the small and unpredictable fraction of iPS cells. By taking advantage of a newly developed single molecule mRNA fluorescence *in situ* hybridization method that can quantify mRNA transcripts of up to three genes in hundreds to thousands of cells, it was demonstrated that the expression of *Esrrb*, *Utf1*, *Lin28*, and *Dppa2* is a better marker for predicting the pluripotent cell than *Fbxo15*, *Fgf4*, and *Oct4*. Although the early phase of reprogramming is accompanied by stochastic gene expression, activation of *Sox2* marks the start of a late phase of reprogramming and initiates a series of consecutive events that lead to pluripotent cell derivation. A hierachical model of gene activation was proposed, and by using downstream factors derived from the later phase, pluripotency was achieved without using the primary Yamanaka factors.

Hans Schöler *(Max Planck Institute for Molecular Biomedicine)*

Hans Schöler was a former Faculty member at Penn Vet, and was the initial Director of the Center for Animal Transgenesis and Germ Cell Research at the University of Pennsylvania. He is currently at the Max Planck Institute for Molecular Biomedicine in Münster, Germany. OCT4 is one of the Yamanaka factors that can transform somatic cells into pluripotent cells. This is the only factor that cannot be replaced by other members of the same family for iPS cell generation. To understand the uniqueness of OCT4, the Schöler lab analyzed its crystal structure. Although protein-DNA contacts were similar to those found for OCT1, the amino terminal part of a linker region is folded as an alpha helix and exposed on the surface of the protein. This was in contrast to OCT1 and OCT6 that contain unstructured linkers. When this region was replaced with that of OCT6, the chimeric molecule lacked reprogramming potential. Point mutations in this region also abolished reprogramming potential. These findings suggest that the OCT4 linker region confers reprogramming potential. OCT4 is thought to have arisen by duplication of the *Pou2* gene during mammalian evolution. Surprisingly, *Oct4* as well as *Pou2* of axolotl was able to reprogram human fibroblasts into iPS cells, despite extensive DNA sequence variation with human *OCT4*. Therefore, pluripotency induction ability is not a feature specific to mammals, but exists in a more primitive vertebrate ancestor.

Fig. 2. Ralph Brinster has received a long list of honors and prizes for his great scientific achivements. (A) *USA President Barack Obama congratulates Ralph Brinster on his* **(B)** *National Medal of Science in 2011.* **(C)** *Professor Brinster receiving the* Doctor Honoris Causa in Medicine *degree and the university Gold Medal from the Rector of the University of the Basque Country (Spain) in 1994.* **(D)** *Ritual procession of that act, accompanied by his academic godfather, Prof. Juan Aréchaga, and the Dean of the School of Medicine and Dentistry.*

John Gurdon *(University of Cambridge)*

The transfer of nuclei from differentiated cells into enucleated eggs results in nuclear reprogramming to a multipotent state. Dr. Gurdon's studies focus on the mechanisms that stabilize differentiation states during embryonic development and how they can be reversed to expand their potential. The identity of lineage-restricted embryonic cells can be remarkably stable. In *Xenopus*, when single prospective endoderm cells from a blastula are transplanted between 2 animal caps that will normally generate the nervous system and skin and cultured, these single cells retain their endoderm identity. Thus, prospective endoderm cells have established their fate by the blastula stage. In contrast, experimental reversal of cell differentiation can be achieved by nuclear transfer. When blastula-stage endoderm nuclei are transplanted into enucleated eggs they express neurectoderm markers but not endoderm markers. Therefore, although the cell is committed, the nucleus can change fate.

Somatic cell nuclear transfer into enucleated eggs which are in 2nd meiotic metaphase can result in DNA replication errors. It is possible to transfer somatic nuclei into growing oocytes in 1st meiotic prophase. Multiple somatic nuclei can be transplanted into the germinal vesicle. This results in intense transcription during culture. By day 4 there is new gene expression. In the mouse, germ cells mature into oocytes in about 17 days. It takes about 10 days after fertilization to differentiate muscle and nerve cells. However, in *Xenopus* oocyte formation lasts about 100 days but it only takes 1.25 days to go from zygote to larva with muscle. Thus, in *Xenopus* the oocyte takes a long time to prepare itself for very quick development.

What are the egg and oocyte components that can reprogram somatic cell nuclei? One component that seems to be important is a histone linker specific to oocytes and early embryos, Hf1oo. This linker histone is rapidly taken up by transplanted nuclei. Dominant negative approaches suggest that Hf100 is an important egg reprogramming factor. Variant histones, actin polymerization and oocyte-derived polymerase II also appear to be important for somatic nuclear reprogramming.

There is a resistance to nuclear reprogramming as cells differentiate. The nuclei from most cells do not readily switch. Transplanted nuclei from early embryonic stages are efficiently reprogrammed but not later differentiated nuclei. Evidence suggests that histone variants and other tightly bound chromatin factors may act as an epigenetic barrier to reprogramming.

Allan Bradley *(Wellcome Trust Sanger Institute)*

Allan Bradley described the introduction of human immunoglobulin loci into the corresponding endogenous mouse loci, creating mouse strains to ultimately generate human monoclonal antibodies for disease therapy. Previous humanized mouse models have suffered from random integrations of transgenes, insufficient size

of constructs to encompass the entire locus, and the generation of mouse-human hybrid antibodies. The new model requires modifications generated by a sequential build of the new loci in ES cells. These extensive and elegant genetic manipulations should result in a new humanized mouse model system to create high affinity human antibodies for therapeutic purposes.

Robert Hammer *(University of Texas Southwestern Medical Center)*

Robert Hammer trained in Ralph Brinster's lab during the early 80's, working on many of the initial pioneering transgenic mouse studies. A focus of the Hammer lab is to understand how defective ribosome biogenesis retards growth and proliferation, yet paradoxically predisposes to cancer. Both of these paradigms occur in the "ribosomopathies", a group of clinically diverse human diseases caused by haploinsufficient mutations in ribosomal proteins or other essential genes required for ribosome biogenesis. To determine the extent to which ribosomal protein S6 (rpS6) is required for regulating growth and proliferation in the liver, they conditionally deleted rpS6, a constituent of the 40S ribosome and target of the nutrient sensor mTOR, during the latter stages of embryonic liver development and in adult liver. Deletion of rpS6 in the developing liver induced severe neonatal hepatic insufficiency by activating p53 in hepatoblasts, which impaired bile duct development and negatively impacted hepatocyte proliferation and survival. Ensuing cholestasis and progressively increasing hepatocyte cell death triggered chronic hepatic progenitor cell (HPC)-mediated regeneration and the activation of multiple pro-growth and -survival signaling pathways, all of which ultimately promoted liver overgrowth and predisposed to liver tumor development. Deletion of rpS6 in adult liver also induced hepatocyte cell death and again triggered HPC-mediated regeneration. These results demonstrate that rpS6 deficiency indirectly generates a permissive environment for tumor development by disrupting hepatic development and homeostasis and forcing the chronic activation of liver stem/progenitor cells and pro-oncogenic pathways to counteract unscheduled cell death and overcome blocked cell proliferation. Importantly, they illustrate the importance of maintaining normal ribosomal dynamics as a means to preserve normal cell and tissue homeostasis and prevent cancer.

Katherine High *(University of Pennsylvania)*

Transgenic approaches to model gene therapies were amongst the first applications of the new transgenic technologies. Dr. High described gene therapy strategies for the *in situ* correction of mutations in the Factor IX locus that is defective in some patients with hemophilia. In a mouse model, zinc finger nucleases were tested *in vitro* for their ability to recognized and cleave the Factor IX locus. These were then introduced into newborn mice with a gene targeting vector to add back a cDNA fragment. Approximately 10% targeting of the endogenous locus was detected. Subsequently, the system was introduced into knockout adult liver cells, achieving alleviation of the hemophilia phenotype. These studies demonstrate the utility of modeling gene therapy approaches in mice for *in situ* corrections of genetic disorders.

Ken Zaret *(University of Pennsylvania)*

Ken Zaret and colleagues examined the initial events in chromatin engagement by transcription factors that reprogram fibroblast cells to pluripotency, as discovered by S. Yamanaka and colleagues. The study revealed that the Yamanaka factors initially bind extensively to distal sites in the genome, including enhancer elements, as opposed to promoters, and do not initially recapitulate the network of binding events that occur in pluripotent cells. Rather, they found large, heterochromatic regions that prevent ectopic transcription factor binding. Genetic studies showed that the presence of H3K9me3 at such regions leads to chromatin structures that impair transcription factor binding, and that disruption of H3K9me3 allows factor binding and greatly enhances the pace of reprogramming. Future studies will be directed to understand how such heterochromatic domains are established in development and how they can be manipulated to allow other types of cellular reprogramming, such as from one differentiated cell type to another.

Michael Brown *(University of Texas Southwestern Medical Center)*

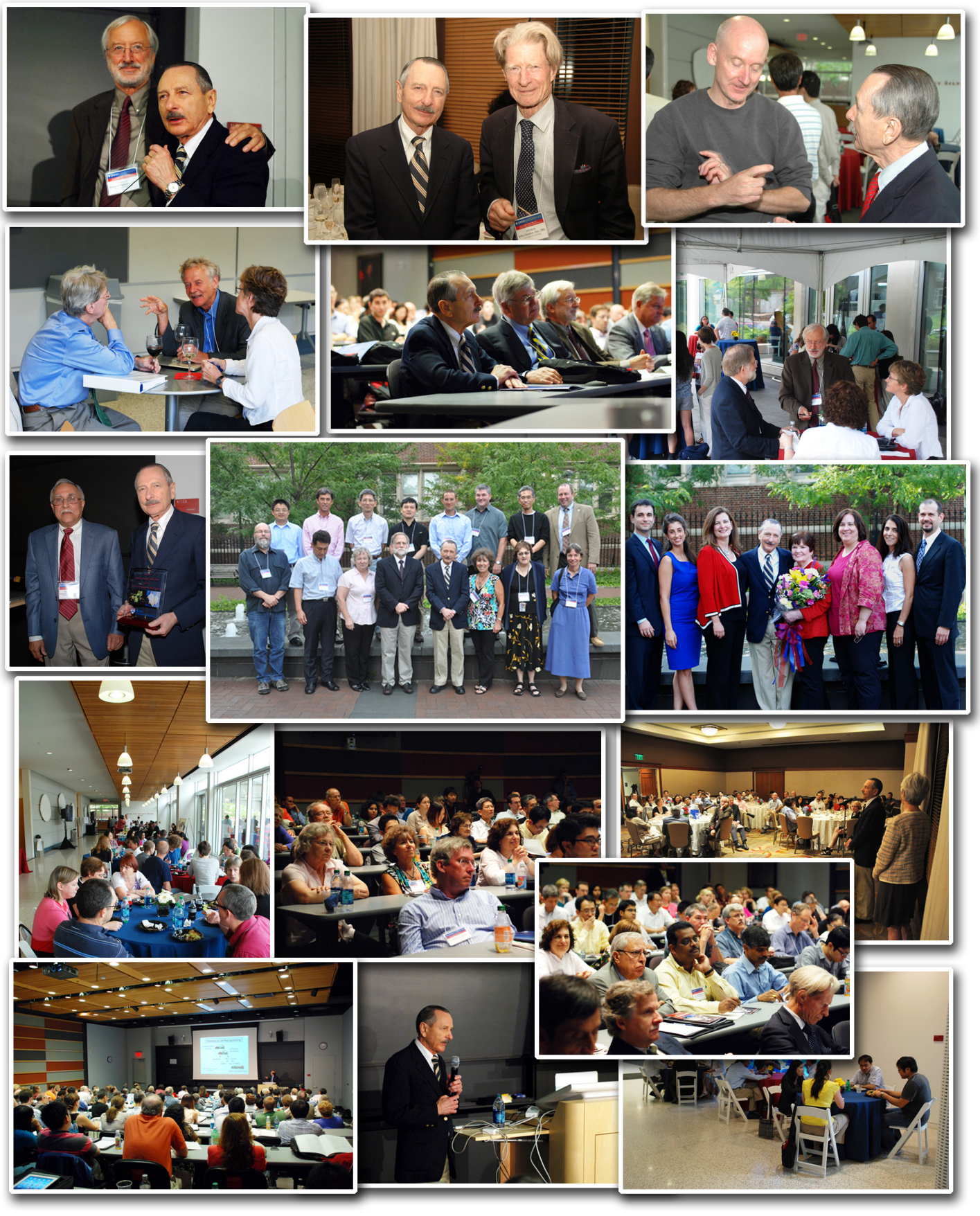
Michael Brown's group with Joe Goldstein and Bob Hammer have created a variety of genetically engineered mice to study the mechanisms that regulate cholesterol metabolism. Among these mouse models, one in particular highlighted how the mouse can open a new door into medicine. Humans with congenital generalized lipodystrophy (CGL) have essentially no subcutaneous body fat because of a defect in adipose cells. They have a greatly distended abdomen because they store fat in their liver. These individuals also become diabetic with increased plasma glucose, insulin, and triglycerides levels. Brown and colleagues were studying the role of sterol regulatory element-binding protein-1 in cholesterol metabolism. SREBP is a B-HLH zip transcription factor with two transmembrane domains that is bound in endoplasmic reticulum (ER) membranes. Through a series of regulated proteolytic cleavages, the bHLH containing fragment is released from the ER to translocate to the nucleus where it binds sterol regulatory elements of enzyme genes involved in sterol biosynthesis. Low levels of sterols leads to the production of the nuclear form of SREBP. High levels inhibit SREBP processing, reducing target gene transcript levels. Thus, SREBP transcriptional activity is sensitive to cholesterol levels through a negative feedback loop.

Overexpression of a truncated form of SREBP that can constituitively translocate to the nucleus in the livers of transgenic mice resulted in greatly enlarged fatty livers although plasma lipid levels were not altered. There was also a progressive decrease in white adipose tissue. Interestingly, overexpression of the truncated SREBP specifically in the adipose tissue of transgenic mice also resulted in an enlarged fatty liver, loss of adipose tissue, insulin resistance and diabetes very similar to CGL. Leptin is normally produced in adipose tissue and inhibits food ingestion. They found that their lipodystrophy mice did not express leptin even with high glucose and insulin levels. They treated their mice with leptin and found that plasma glucose and insulin levels normalized. They wondered if leptin could be used to treat lipodystrophy patients. A clinical trial was performed with collaborators from the NIH, finding that leptin treatment alleviated the lipodystrophy in humans. This is a remarkable example of how mechanistic findings from transgenic mouse studies can inform medicine, resulting in a successful therapy for human disease.

25 August 2012

James Thomson *(University of Wisconsin)*

James Thomson received his V.M.D./Ph.D. from the School of Veterinary Medicine at the University of Pennsylvania. He described



human embryonic stem (ES) cell and iPS cell derivation. Although human ES cells were originally cultured in complex medium, a new serum-free culture medium consisting of only insulin, FGF2, NODAL, and TGFb1 was developed to maintain human ES cells in an undifferentiated state without feeder cells. Through the course of this study, it was discovered that bovine serum albumin, which is a commonly added medium component, is dispensable for ES cell culture. Of the several critical media components, FGF2 is a critical factor for human ES cell self-renewal. It not only promotes self-renewal of human ES cells, but also activates the MEK-ERK pathway in differentiating cells and maintains *Nanog* expression, which influences the differentiation pathway. Although *Nanog* is not only involved in the establishment and maintenance of pluripotency, this gene is also involved in lineage determination of ES cells, as cells exit the pluripotent state. ERK activation also induces phosphorylation of OCT4 and weakens its DNA binding activity, which decreases reprogramming efficiency. These results underscore the complexity and importance of the FGF2 pathway in regulating ES cell maintenance and differentiation.

Ina Dobrinski *(University of Calgary)*

Ina Dobrinski trained in Ralph Brinster's lab in the late 90's/early 00's. She then initiated her independent research in the Center for Animal Transgenesis and Germ Cell Research at the University of Pennsylvania and is currently at the University of Calgary. In her own lab she developed a novel testicular tissue graft paradigm for spermatogenesis. She described the use of the testicular tissue grafting method for examining chemical toxicity on reproductive tissues. Subcutaneous transplantation of immature testis fragments from pig or primate in immunodeficient mice results in graft-derived spermatogenesis. This technique was used to study chronic exposure of the infant primate testis to phthalates, which are chemicals widely used worldwide as plasticizers for polyvinyl chloride (PVC) and other plastics. Phthalates increase plastic flexibility and soften their mechanical properties. Exposure to phalate ester during pregnancy not only reduced the testosterone concentration but also increased congenital malformations of the male reproductive tract, including smaller seminal vesicles. Spermatogenesis was inhibited at high concentrations. These experiments provide the foundation for systematic studies of the effects of suspected toxicants like phthalates on reproductive tissue and organ development.

Richard Schultz *(University of Pennsylvania)*

Richard Schultz described how an increase in aneuploidy occurs in meiotic cells of aging females. Using naturally aging mice as a model system, it was found that spindle abnormalities and faulty chromosome congression on the metaphase plate are associated with advanced maternal age and likely contributes to the observed increased incidence of aneuploidy. Expression profiling of global gene expression was carried out using oocytes obtained from young and old mice. It was found that aged oocytes exhibit misexpression of many genes, including genes involved the spindle assembly checkpoint (SAC), chromosome congression and attachment to kinetocore microtubules, and spindle assembly. In addition, the normal pattern of degradation of maternal mRNAs was not observed following maturation of aged oocytes, and levels of the meiotic cohesin protein REC8, the meiotic counterpart of mitotic cohesion protein, were significantly reduced on chromosomes from old mice. Therefore, deterioration of cohesion is a possible cause of age-related aneuploidy.

Marisa Bartolomei *(University of Pennsylvania)*

Marisa Bartolomei described the regulation of genomic imprinting at the *H19-Igf2* locus, which depends on a differentially methylated domain (DMD) acting as a maternal-specific, methylation-sensitive insulator and a paternal-specific locus of hypermethylation. Although recent studies revealed that DNA methylation requires a favorable histone environment, it is still unclear what sequence signature distinguishes DMDs from other CpG rich regions in the genome. CTCF is thought to bind to four repeats of unmethylated DMD on the maternal chromosome, and blocks the expression of *Igf2*. On the other hand, CTCF cannot bind to methylation DMD on the paternal chromosome, which allows expression of *Igf2*. She has examined the impact of genetic elements *in vivo* by creating mutant mice with several mutations by gene targeting. Mutations in only 9 of 62 CpGs in the DMD induced an epigenotype switch of the paternal region to that of the maternal region. In addition, removal of the four repeats of CTCF biding sites led to biallelic *Igf2* expression, which resulted in development of larger embryos. Detailed analyses of the space and pattern of the CTCF binding sites were carried out. Mice without the second and third repeats grew slightly larger than normal but also had a large tongue due to increased *Igf2*. Because imprinting in the liver was maintained, it was concluded that imprinting in mesoderm was abnormal. The challenge remains to unravel the mechanisms by which the DMD is methylated and regulated in the germline.

Janet Rossant *(The Hospital for Sick Children, Toronto)*

Janet Rossant described the role of the Hippo signaling pathway in early mouse embryo development. The first cell lineage to be specified in the preimplantation embryos is the trophoectoderm. The key molecule involved in trophoectoderm (TE) differentiation is *Cdx2*. This gene is expressed at varying degrees in all blastomeres starting at the eight-cell stage, and becomes restricted to cells located peripherally in the late morula, which will become future TE cells. Embryos without *Cdx2* do not develop normally due to defective development of TE. It is still unknown how Cdx2 expression is activated. However, expression of *Yap* and *Tead4* in cells located peripherally was shown to cooperatively induce *Cdx2*. Specific expression of *Cdx2* was caused by selective expression of *Yap* in peripheal cells, because *Tead4* is distributed in all cells of the embryos. Phosphorylation of YAP by Hippo pathway kinase large tumor suppressor homolog 1 and 2 (LATS1 and 2) prevents its nuclear localization in the inside cells of the embryo. Mouse embryos lacking both *Lats1* and *Lats2* genes failed to restrict YAP to the cytoplasm of inside cells. After TE differentiation, the ICM segregates into the epiblast (EPI) and primitive endoderm (PE). This process is influenced by FGF levels. Adding an FGF receptor inhibitor converts all eight cells into *Nanog*-expressing pluripotent cells, and promotes ES cell derivation. Moreover, embryos lacking any of the FGF4-FGF receptor2 pathway components cannot differentiate into PE cells. Although the mechanism by which this pathway induces only some of the cells to become PE is not known, these data suggest that this pathway activates PE cell fate. In humans, however, adding an inhibitor to FGF receptor does not inhibit PE formation, suggesting that other molecules are involved. In this context, it is noteworthy that mouse embryos without *Porcupine*, which acetylates Wnt proteins, cannot complete gastrulation and

remain in an EPI state. Considering the male lethality of human *Porcupine* mutants, it was suggested that this signaling pathway may play a role in commitment to PE.

Takashi Shinohara *(Kyoto University)*

Takashi Shinohara trained as a postdoctoral fellow with Ralph Brinster in the late 90's/early 00's, examining conditions for spermatogonial stem cell culture and expansion. In his own lab he has continued studying spermatogonial stem cell biology. Stem cells are thought to reside in a special microenvironment called niche, which provides self-renewal factors. He described a method for reconstituting the germline niche in vitro. When spermatogonial stem cells (SSCs) were plated on top of testis feeder cells, they migrate beneath the feeder cells and produced colonies with a cobblestone morphology, which were indistinguishable from those produced by hematopoietic cells beneath bone marrow stroma cells. Because cells in these cobblestone colonies maintained SSC activity, it was suggested that dissociated testis feeder cells reproduced niches *in vitro* and attracted SSCs. Cobblestone colony formation was inhibited by interfering with glial cell line-derived neurotrophic factor (GDNF) or CXCL12 signaling, which suggested that these molecules are mediating SSC homing into the germline niche. This idea was confirmed *in vivo* by using a dominant negative GDNF receptor mutant or using CXCL12 receptor knockout mice. Moreover, GDNF upregulated CXCL12 receptor expression in cultured SSCs, suggesting that they act synergistically to promote SSC homing. These molecules identify CXCL12 and GDNF as SSC homing molecules.

Ralph Brinster *(University of Pennsylvania)*

Ralph Brinster gave the closing remarks, and provided a historical perspective on research on mammalian embryo culture and transgenesis. He emphasized the importance of studying germline cells for improving the health and life of animals and man. He also stated that transgenesis was the most recently developed method by which humans interacted with their environment. This happened after the domestication of plants and animals, selection and breeding for desirable traits, and characterization of genetic elements. All of these steps were necessary for the modification of individual genes, which became possible in the last thirty years. Although traditional methods for transgenesis were based on the manipulation of zygotes, spermatogonial transplantation, which he developed in 1994, has created new possibilities. SSCs from several animal species can be cryopreserved, cultured, and genetically modified for animal transgenesis. Clinically, this technique will be useful for the preservation of fertility of prepubertal boys receiving cancer treatments, which often make them infertile. Future developments, such as *in vitro* spermatogenesis, conversion of somatic cells to SSCs, and culturing and genetically modifying SSCs from diverse animal species, will make these cells a powerful vehicle for transgenesis.

Epilogue

An exciting question, but one that is impossible to answer, is in what new directions will this field and area of science evolve? One only need think back 10 years, 20 years or 50 years to know how naïve scientists were in anticipating dramatic events that would occur in our knowledge and in our ability to manipulate and understand the germline and stem cells, as well as the processes that go into the development of an animal. If one is so brave as to anticipate possible developments, these might include the following:

1. A fundamental and revolutionary change in our interaction with evolution and with other species will be the conversion of somatic cells to germ cells. A cell conversion that is likely to occur soon, within a few years, will be the generation of spermatogonial stem cells from somatic cells. A more difficult reprogramming will be involved in developing an oocyte from a somatic cell, and finally the generation of zygote using *in vitro* differentiated germ cells that might develop into an offspring.

2. It seems reasonable to expect that in the not-too-distant future genetic changes will be able to be introduced into somatic cells, and likely into germ cells, without leaving behind a footprint. Such an advance will provide an important step toward the production of somatic cells and germ cells from induced pluripotent stem cells for regenerative medicine.

3. Unique studies, likely involving three-dimensional structures, that will allow one to produce the differentiation process *in vitro* in order to understand the regulatory mechanisms involved are certainly in the future. These differentiation experiments will provide a unique opportunity to understand the complexity involved in generating a complete organ, and will lead likely to the development of artificial organs for replacement in the body.

4. The possibility of correcting genetic defects in humans through intervention in the germ cells and the developing embryo can be foreseen. In addition, targeting of genetic defects and their correction is likely to be possible in fetuses and adults.

5. Some developments will prove extraordinarily challenging. These are likely to include complex processes. For example, the generation of the uterine environment and its regulation to produce pregnancies outside the body is a monumental task. Perhaps the greatest hurdle in our understanding of the body will involve the brain. Its complexities and interactions are incredibly diverse, and we know little about them.

Clearly, there are many other areas which might be discussed and of course progress in the future can never be anticipated. That is the very nature of scientific discovery.

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Further Related Reading, published previously in the *Int. J. Dev. Biol.*

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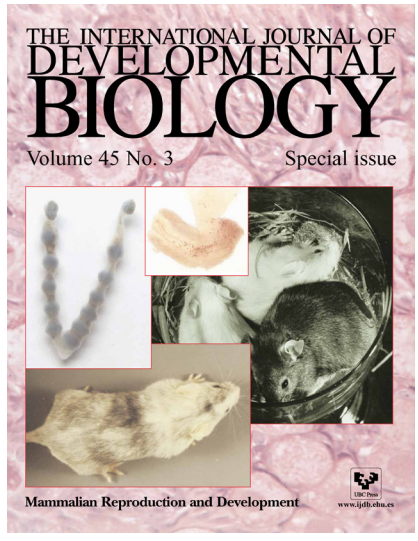

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