Volume 4, Issue 1
April 2005

Texas A&M University
CERH Highlights

What's Inside...

Protein Technologies Facility Core

Genomics Facility Core

Texas Forum on Female Reproduction

CERH Annual Symposium

Community Outreach and Education Program

Pilot Projects

Research Highlight

FROM THE DIRECTOR

by Philip E. Mirkes, Ph.D.

Greetings to all CERH members:

2004 is now behind us, so I thought it would be a good time to look back at what we have collectively accomplished in the past year, and then briefly highlight what lies ahead for 2005.

First, I want to highlight several CERH members who were selected for awards in 2004. Dr. Fuller Bazer received the Carl G. Hartman Award from the Society of the Study of Reproduction and was appointed Texas A&M University Distinguished Professor. Dr. Richard Finnell was appointed Texas A&M University Regents Professor. Dr. Susan Golden was appointed Texas A&M University Distinguished Professor and was named a Fellow of the American Academy of Microbiology. Dr. Wallace Mckeehan was appointed Texas A&M University Regents Professor. Dr. Marcia Ory received the Society for the Scientific Study of Sexuality Hugo G. Beigel Research Award and the International Council on Active Aging Industry Innovator Award. Dr. Tim Phillips was selected as the recipient of the Bush Excellence Award for Research. Dr. Steve Safe was selected as the 2004 recipient of the JoAnn Treat Research Excellence Award by the Texas A&M Research Foundation. Dr. Thomas E. Spencer was the recipient of a Sigma Xi Young Investigator Award and the American Society of Animal Science Young Animal Scientist Award-Research. Dr. Marinna Vannucci received a National Science Foundation CAREER Award and was the recipient of the Mitchell Prize from the International Society for Bayesian Analysis. Congratulations to all.

In October, many of the CERH members gathered at the Del Lago Resort and Conference Center for a Center retreat. On the basis of comments I received at and after the retreat, I think it is safe to say that the retreat was a success and that not only did we come away with a better sense of what the Center has been but also what we want it to become. I want to thank everyone who contributed to the success of this retreat and to announce that the Center retreat will be an annual event.

Be sure to cite Center support (P30-ES09106) on all relevant publications.

Taking ideas and suggestions generated during the Center retreat to heart, the Scientific Advisory Group (SAG) approved several Center initiatives. One of these is the formation of a new facility core, the Biostatistics/Bioinformatics Facility Core, under the leadership of Raymond Carroll. This new core will provide access to statistical support on the one hand and bioinformatics support on the other to CERH investigators. With respect to the latter, the Center is seeking to hire a full-time bioinformaticist. Another initiative involves the Analytical Facility Core, to which we have now added access to HPLC analysis of amino acids and other small nitrogenous molecules, thanks to the addition of Dr. Guoyao Wu's expertise to this core. Other initiatives include consolidating the Genomics Facility Core, under the leadership of Robb Chapkin, and the Transgenic Facility Core, under the leadership of Jim Martin, and adding needed equipment to several of the facility cores. Finally, we have reorganized what was the Reproductive and Developmental Biology Research core into two separate research cores, the Reproductive Biology Research Core, under the leadership of Tom Spencer, and the Developmental Biology Research Core, under the leadership of Rick Finnell.

In early December, we presented a summary of these changes and our vision for the future of the CERH to our External Advisory Board (EAB). We received very positive feedback from the board members while they were on campus, and I have just received a copy of their report, which is also uniformly positive and includes several potentially useful suggestions. The SAG will study this report and determine what additional initiatives we need to take to maximize the Center's effectiveness in carrying out its mission. I want to take this opportunity to thank the External Advisory Board members (Daniel Acosta, Dennis Bier, David Eaton, and Roger McClellan), the Research and Facility Core Directors, COEP staff, and the CERH administrative staff for all their hard work in making this a successful EAB meeting.

After the EAB meeting, we proceeded to the annual CERH symposium, entitled "Dietary Fiber and Colon Cancer," which featured outstanding presentations from Drs. Sheila Bingham (MRC Dunn Human Nutrition Unit), Elaine Lanza (NCI), and Ross Prentice (Fred Hutchinson Cancer Research Center). The symposium was followed by an active discussion period and concluded with poster presentations (42) from CERH and TAMU investigators. I want to thank Joann Lupton and Robb Chapkin, who organized this symposium, and the CERH administrative staff (Kim Daniel, Gail Goolsby, Ashley Riggs, and Amber Robinson), who made it all happen.

In conclusion, the Center has had a productive year and I thank all of you. Now, what lies ahead? Besides our usual Center activities, this year we have an added task: to prepare the CERH competitive renewal proposal (due February 1, 2006, less than 12 months away!). At the next SAG meeting, we will finalize the time table for the months leading up the site visit, probably in May of 2006. When the timetable is ready, I'll pass it on to you. As we begin to collect materials for the Center renewal, I would like to ask all CERH members to provide requested information in a timely manner. I'd also like to introduce the Center's new Program Coordinator, Anna Kjolen. Amber Robinson has left the Center to be a full-time mom to Abigayle Hope, born on March 10. Congratulations Amber and Justin!

Again, thank you all for your contributions to the Center that made 2004 a successful year! I look forward to working with all of you in 2005.

Phil Mirkes

PROTEIN TECHNOLOGIES FACILTY CORE

The Protein Technologies Facility Core has been very active with workshops and acquiring new instrumentation to expand the scope of protein analysis services. The PTFC hosted another very successful workshop on 2D Gel Electrophoresis and Proteomics in early November. The workshop was attended by 13 CERH scientists who learned how to prepare samples for 2D gel analysis using Immobilized pH Gradient DryStrip Technology from GE Healthcare. All students extracted proteins, ran and stained gels and tried to identify proteins using modern mass spectrometry-based techniques. This workshop will be offered again in late Spring/Early Summer for those of you who missed it. CERH member labs are provided with scholarships to defray the costs of tuition.

The PTFC is adding new technologies and accessories this spring in order to provide a wider range of protein-related services. A nanospray source has been acquired for the ThermoFinnigan LCQ DecaXP LC/MS ion trap mass spectrometer and is being installed this week. It will be used to increase the sensitivity of in gel digestion experiments and enable the PTFC to identify lower abundance proteins from Coomassie, Sypro and Silver stained gels. The attachment will also be applied to protein quantification experiments using ICAT technology. This technique was developed at the University of Washington by Ruedi Abersold and is used by Dr. Phil Mirkes here at the CERH to quantitate and identify proteins using specially designed alkylating agents. The PTFC will post a description of the ICAT system on our website (pcl.tamu.edu) so you can read more about it. All these improvements come at the request of faculty at TAMU and the CERH and are made possible by financial support from the CERH. We hope you will learn more about these opportunities in protein characterization and visit us to discuss your research needs.

GENOMICS FACILTY CORE

In December, the Genomics core held a CodeLink Microarray Award competition. Several labs applied for the award of 6 free CodeLink Genome microarrays complements of GE and free array processing complements of the Genomics core. Three awardees were selected from the excellent proposals. The awardees were the labs of Drs. Weston Porter, Yanan Tian and Alan Parrish. Congratulations to these investigators! We plan to hold this competition again at the end of the year. Please visit the Genomics webpage for information on CodeLink microarrays (http://cerh.tamu.edu/facility_cores/dna_tech/)

TEXAS FORUM ON FEMALE REPRODUCTION

The eleventh annual meeting of the Texas Forum on Female Reproduction (TFFR) sponsored by the Texas Womens Reproductive Health will be held at the Institute of Biosciences and Technology (IBT) in the Texas Medical Center, Houston on April 14-15, 2005. The Forum is a two day meeting that has both oral and poster presentations with a focus on female reproduction. It is attended by scientists and clinicians from institutions in southeast Texas and every year, brings together about 115 people. In previous years, the forum has been well attended by trainees and PIs from the CERH Reproductive and Developmental Biology Research Core.

Last year, Dr. Safe, presented the keynote lecture. This year, two plenary speakers have been announced including Mark Hughes, M.D., Ph.D., Genesis Genetics Institute and Wayne State University who will present the lecture “Preimplantation Genetic Diagnosis - Technology, Medicine, & Ethics,” and Jonathan Tilley, Ph.D., Massachusetts General Hospital and Harvard Medical School who will present “Germline Stem Cells and Follicular Renewal.” The advance registration deadline is March 14, 2005. Additional information and registration & abstract forms available at http://www.utmb.edu/twrhc. The sponsors of the meeting are counting on continued strong participation in this meeting by CERH members.

CERH ANNUAL SYMPOSIUM

The CERH Scientific Symposium was held on December 15, 2004. The goal of the symposium was to understand the issues associated with establishing the role of a dietary component on disease prevention. The invited speakers would be describing the problems in determining whether humans benefit from elevated levels of dietary fiber by having reduced risks of colon cancer, an timely and controversial issue.

The speakers were Dr. Sheila Bingham from the Medical Research Council Dunn Human Nutrition Unit in Cambridge, England, Dr. Elaine Lanza from the National Cancer Institute, Bethesda, and Dr. Ross Prentice from the Fred Hutchison Cancer Center, Seattle, Washington. Dr. Bingham described the results obtained from the EPIC study, in which one of the goals was to determine the incidence of colon cancer in various European Union countries and the consumption of dietary fiber in those populations. Dr. Lanza discussed the NCI Fiber and Polyp Recurrence Trial that used dietary fiber supplements to suppress the recurrence of colon polyps. Dr. Ross Prentice gave an overview of the statistical problems associated with performing clinical trials to understand the relationship of diet and cancer prevention. After the speakers had given their formal presentation, our own Dr. Robert Chapkin and Dr. Raymond Carroll gave a short presentation to stimulate discussion before opening up the floor to a panel discussion.

The symposium closed with presentation of the awards for the best Post-doctoral and Graduate Student Posters. The award for best Post-doctoral poster was given to Dr. Kimberly Drews for her poster on "A Likelihood Based Approach to the Analysis of Colonic Crypt Signaling". The awards for best Graduate Student posters were given to Valentina Massa, Scott Angell, Jeong-Eun Lee, Tety Leonardi, and Michael Vitalini.

COMMUNITY OUTREACH AND EDUCATION PROGRAM

Dr. Nancy Valdez joined the Community Outreach and Education Program as a research scientist on Feb.1, 2005. Having significant prior experience through the Texas Cooperative Extension in developing nutrition programs targeting underserved populations, she will be responsible for initiating a variety of nutrition modules for COEP. Dr. Vivas-Valdez received her Master's and Ph.D. degrees in Food Science and Technology from Texas A&M University. She is originally from Mexico, where she graduated with a Bachelor's degree in Biochemical Engineering of Foods from the Instituto Tecnológico de Mérida. Nancy worked for Texas Agricultural Extension at Texas A&M as an Extension Associate for the Better Living for Texans program. During her work for this program, she gained experience in nutrition as applied to disparity groups. She contributed to the development of the Bridge to Health/Puente de Salud curriculum, which targets Mexican American Women. Dr. Vivas-Valdez was hired as Research Scientist in July 2004 by the Center for the Study of Health Disparities at Texas A&M to provide research assistance to the PIs of various cores of the Center's grant.

The Center for Environmental and Rural Health brought smiles to the faces of twenty-one families in Rio Bravo this past holiday season. As participants in Dr. K.C. Donnelly's pesticide exposure study, each of these families was presented with certificates for groceries. In addition, a total of forty children from the Rio Bravo colonia received donated gifts from the generous CERH and SRPH faculty and students. Two Giving Trees were set up with tags bearing the names of the children for the purpose of collecting anonymous donations. Brian R. Smith, the Director of Region 11 of the Texas Department of Health, brought additional cheer by graciously playing the role of Santa Claus, driving the CERH Mobile, and posing for photographs with the children.

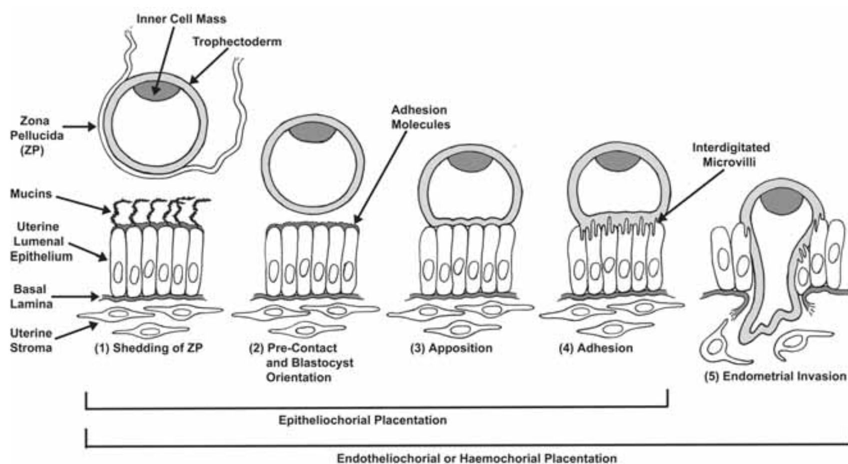
PILOT PROJECT RESEARCH

Gregory Johnson, Ph.D.

Unexplained chronic infertility currently affects more than six million women in the USA. Although this represents much emotional pain for couples trying to begin families, it is not a surprising statistic in light of the fact that even in fertile women only 40 to 50% of babies are estimated to survive beyond the fifth month of pregnancy. Three quarters of these pregnancy failures are not even clinically recognized as pregnancies because of an inability of the developing embryo to attach to the uterine mucosal surface, implant into the uterine wall, or form a functional placenta. Therefore, demand is great in the medical community for the identification of useful molecular and cellular markers of uterine function and receptivity to the embryo.

The long-range goal of our research is to understand the physiological, cellular and molecular interactions between the developing embryo and its associated placental membranes, and the maternal uterine endometrium that are essential for the establishment and maintenance of pregnancy. Our ultimate goal is the application of new knowledge towards clinical strategies to prevent pregnancy loss in companion animals, domestic livestock, and women.

Embryo implantation involves five potential phases of increasingly complex interactions between the embryo and the uterine wall (Figure 1). This process requires pregnancy-specific alterations in extracellular matrix (ECM) at the embryo-maternal interface, and disruption of this process is recognized as a significant contributing factor to infertility in women. ECM has direct adhesion and signaling roles in embryo attachment, invasion, and development. Our laboratory has utilized pigs, sheep, goats and mice to focus on the ECM protein osteopontin. We are interested in osteopontin because the human uterine mucosal surface (or lumenal epithelium, LE) and decidua of early pregnancy express osteopontin, a molecule involved in cell-cell and cell-ECM communication, cell proliferation/migration/survival, and regulation of cytokines involved in tissue remodeling. Indeed, osteopontin is prominently expressed at the embryo-maternal interface of many mammals, including primates, ruminants, pigs, rabbits and rodents. In mice, disruption of the osteopontin gene results in decreased pregnancies at mid-term, and embryos that are significantly smaller than wild-type counterparts at term. We believe that if advances in improving embryonic survival are to be realized, it is critical to define the physiological role of molecules such as osteopontin in regulating the progressive changes at the embryo-maternal interface that ensure successful pregnancy.



Recently we have shown that osteopontin expression on the mucosal epithelial surface of the pig uterus, the surface that the embryo will attach to for implantation, is induced by embryonic secretion of the steroid estrogen. Indeed, in situ hybridization (Figure 2) indicates that osteopontin

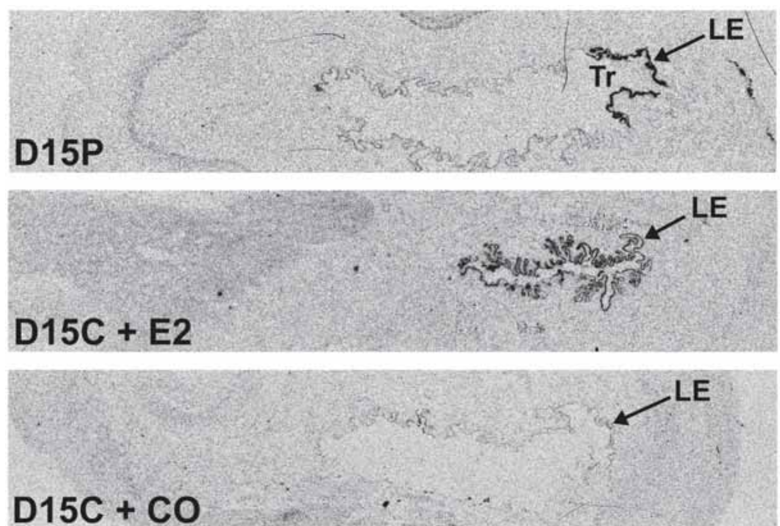


Figure 2. In situ hybridization for osteopontin mRNA in porcine uterine cross-sections. The black lines indicate hybridization of antisense porcine osteopontin cRNA probes to mRNA in uterine lunenal epithelium (LE). Tr, embryonic trophectoderm.

increases in discrete regions of the uterine lumenal LE in close proximity to the embryo (trophectoderm, Tr) by Day 15 of pregnancy (P), just as the embryo is orienting, apposing and adhering to this surface for implantation. Systemic injection of estrogen (E2) but not corn oil control (CO), to mimic secretion of estrogen from the conceptus during normal pregnancy recognition in pigs, resulted in up-regulation of osteopontin throughout the uterine LE. Clearly environmental estrogens have the potential to disrupt the early stages of implantation in pigs, and perhaps other species including women.

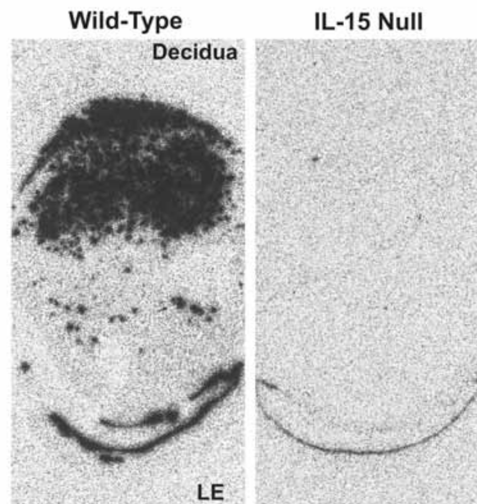


Figure 3. In situ hybridization for osteopontin mRNA in mouse uterine cross-sections. OPN mRNA is present in both the decidual natural killer (NK) cells and lumenal epithelium (LE) of wild type mice, but is not present in the decidua of IL-15 null mice that lack mature NK cells.

Currently our laboratory is investigating the expression of osteopontin in the mouse uterus. The osteopontin gene ablated mouse exhibits reproductive phenotypes consistent with implantation and placentation defects. With this model we will be able to test the hypotheses that osteopontin in the uterine mucosal epithelium mediates conceptus attachment to the uterine wall during implantation, and that osteopontin in decidua is essential to support normal fetal/placental growth and development. Preliminary results indicate that osteopontin is present on the apical surface of uterine LE when the conceptus attaches to the uterus, and is later present in the decidua that surrounds the conceptus after invasion into the uterine wall. Moreover, mice that lack interleukin (IL)-15, a protein required for maturation of uterine decidual natural killer (NK) cells, do not express osteopontin in their decidua. In situ hybridization for osteopontin showed significant hybridization in the decidua of wild-type, but not IL-15 null mice. Therefore osteopontin is expressed by NK cells that are known to profoundly affect placental support of embryonic development through immune and angiogenic mechanisms. We hope this research will provide fundamental new knowledge for use in development of strategies to ameliorate uterine dysfunction that contributes to infertility in women.

RESEARCH HIGHLIGHT

Warren Zimmer, Ph.D.

The research in my laboratory centers on understanding mechanisms that regulate the expression of specific genes during development. We began looking at factors that influence the smooth muscle component of the gastrointestinal (GI) tract utilizing the avian gizzard as a model. It has been known for some time that intestinal smooth muscle cells are predominately derived from mesodermal precursors, however the factors regulating the smooth muscle myogenic pathway are not understood. We have examined the regulation of the Smooth Muscle Gamma Actin (SMGA) gene in developing avian gizzard as a model of visceral mesoderm development. We demonstrated a definite lineage of cells committed to the smooth muscle pathway in visceral muscle and that the terminal differentiation of these cells is dependent upon expression of the trans-factor Serum Response Factor (SRF).

Since SRF is known to work with other proteins, called partner proteins or co-activators, to engage cell specific transcriptional responses, current experiments in the lab are focused upon determining the identity of these proteins and determining how they work with SRF to achieve smooth muscle terminal differentiation. We initiated these studies looking at the homeodomain proteins Nkx 3.1 and Nkx 3.2, which are mammalian homologs of *Drosophila bagpipe*, a protein important for visceral mesoderm differentiation. We demonstrated that both of these proteins are capable of collaborating with SRF to regulate SGMA transcription. We isolated the mouse genes encoding these proteins and in collaboration with Robert J. Schwartz (IBT) we have constructed knockout mice lacking the expression of these regulatory proteins, and NICx 3.1 is known to be an important regulator of prostate development and are currently characterizing the phenotypes of homozygous knockout animals for Nkx 3.1 and Nkx 3.2.

While Nkx 3.1 expression is detected in a variety of embryonic tissues, including the somites, vascular smooth muscle, and regions of the central nervous system, its expression is androgen regulated and restricted to the male urogenital tract, predominately within the prostate, in both mouse and man. Nkx3.1 is known to be an important regulator of prostate development and we believe that it exhibits properties of a tumor suppressor of prostate adenocarcinoma. We have identified SMGA as the first known molecular target for Nkx 3.1. More recently, we showed that SMGA expression is regulated in prostate epithelia via Nkx 3.1/SRF interactions at a novel *cis* element we refer to as the Nk/CArG (Figure 1). Therefore, we have described the first biologically appropriate target for Nkx 3.1 regulatory activity in prostate epithelial cells. Recently, we have examined SMGA expression in prostate epithelia using immunocytochemistry. As illustrated in Figure 2 we observe a tight correlation with increased SMGA expression and cancer progression as measured by Gleason Score and cellular morphology. We are now using *in vitro* and *in vivo* approaches to examine how Nkx 3.1 and/or SRF is responsible for altered prostate cell function.

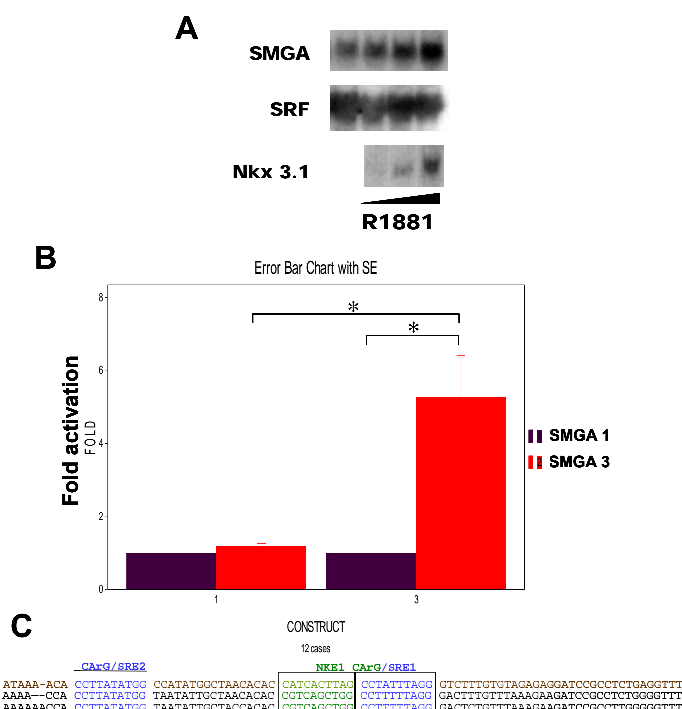


Figure 1. Regulation of SMGA mRNA and Transcription in Prostate Adenocarcinoma cells. A. Northern Blot analysis of SMGA, SRF, and Nkx3.1 RNA in the Androgen responsive LNCaP adenocarcinoma cell line. There is increased SMGA mRNA in cells stimulated with the synthetic androgen R1881, likely due to the direct androgen regulation of Nkx3.1 gene transcription. B. SMGA promoter-receptor genes are transcriptionally-activated in LNCaP cells treated with androgen, only when the binding sites for Nkx3.1 and SRF are present such as the HSMGA3 construct. C. Sequence homology of the chicken (top), human (middle), and mouse (bottom) promoter segments that are equivalent to the HSMGA3 construct.

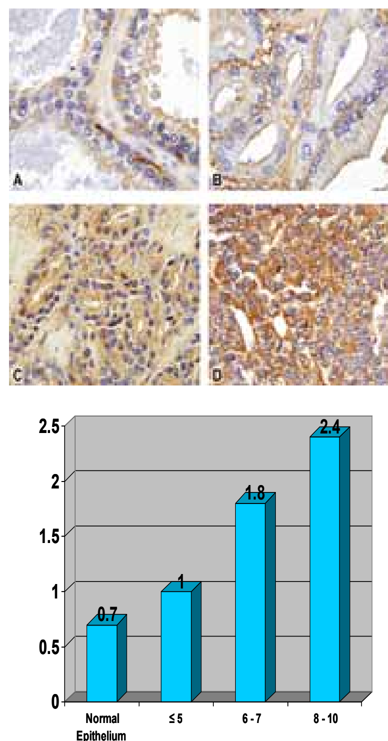


Figure 2. Immunocytochemical localization of SMGA in prostate tissue. A-D show prostate tissue at increased severity of adenocarcinoma stained with the actin-monoclonal antibody HUC 1-1. In normal epithelia (A) SMGA is localized to the apical boundaries of the cell although plasma membrane staining is well defined. This polarization of SMGA localization breaks down with cancer severity (B-D). SMGA expression was quantified and plotted as a function of Gleason Score, or severity of cancer. There is increased SMGA staining in prostate epithelia as they become more aggressive adenocarcinomas.

Homozygous mice deficient in Nkx 3.2 expression exhibit skeletal dysplasia and anomalities of the midgut section of the GI tract. The skeletal dysplasia is exhibited by severe malformation or absence of veterbral column and cranial bones of mesodermal origin (Figure 3). Based on our work with visceral mesoderm, we suggest that the molecular basis of Nkx 3.2 regulation of axial skeleton differentiation occurs via interactions with SRF, which provides functional regulatory capability by activating gene programs of developing bone.

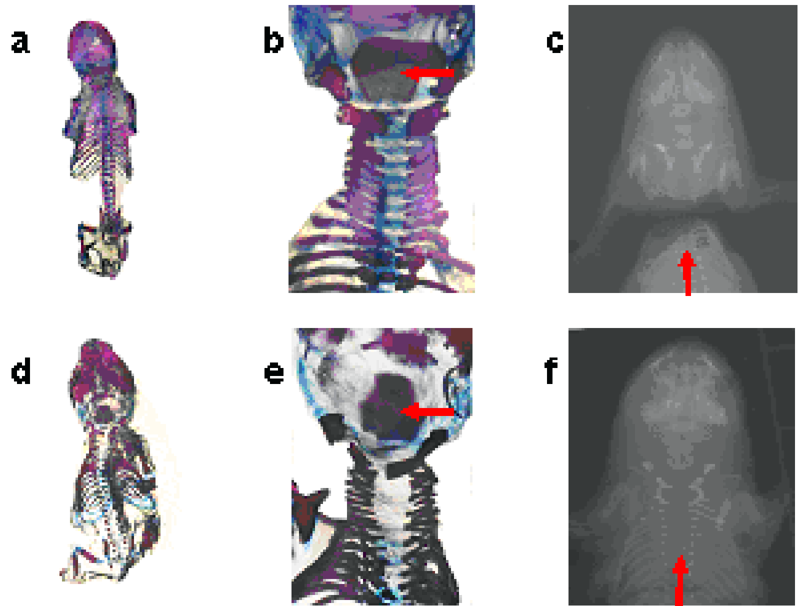


Figure 3. Skeletal dysplasia in Nkx3.2 knockout mice. Skeletons from wild-type (A-C) and homozygous knockout Nkx3.2 18.5 day embryonic mice (D-F) were examined by alcian blue and alizarin red staining (A, B, D, E) and X-ray (C, F). The Nkx3.2 knockout mice exhibit vertebral column developmental problems as well as complete loss of certain cranial bones, such as the basioccipital (arrow) and basiosphenoid bones.

We are assaying the temporal and spatial patterns of Nkx 3.2 and SRF expression, examining the consequences of tissue-specific ablation of Nkx 3.2 and SRF expression, and utilizing DNA array along with subtractive hybridization techniques to identify targets of Nkx 3.2/ SRF regulatory activity in developing bone. Examining the midgut segment of homozygous mutant Nkx 3.2 mice revealed that there is no pyloric sphincter muscle formed and the epithelia of the GI tube are not differentiated. From these results we developed a model that suggests the formation of the stomach sphincter muscles provides spatial cues to the developing epithelia to differentiate into gastric (stomach) or absorptive (intestine) phenotypes. We are developing methods – *in vitro* and *in vivo;* using both the whole embryo cultures and transgenic mice - to analyze these cues and determine the molecular mechanism(s) that allow them to activate appropriate mucosal epithelial development. These studies will provide new information regarding mechanisms and signaling cascades that govern GI tract and skeletal development as well as provide insights into congenital defects and conditions that affect these developmental processes.

CERH Contact Information

Administration

Director: Philip E. Mirkes
pmirkes@cvm.tamu.edu

Research Core Directors

Biostatistics and Community Health:
Raymond Carroll, carroll@stat.tamu.edu

Chemical Biology:
Stephen Safe, ssafe@cvm.tamu.edu

Nutrition:
Joanne Lupton, jlupton@tamu.edu

Reproductive Biology:
Tom Spencer, tspencer@ansc.tamu.edu

Developmental Biology:
Richard Finnell, rfinnell@ibt.tamu.edu

Community Outreach and Education

Carmen Sumaya,
csumaya@cvm.tamu.edu

Pilot Projects

Robert Chapkin,
r-chapkin@tamu.edu

Facility Core Directors

Analytical Services:
Kirby Donnelly, kdonnelly@cvm.tamu.edu

Biostatistics and Bioinformatics:
Raymond Carroll, carroll@stat.tamu.edu

Genomics:
Robert Chapkin, r-chapkin@tamu.edu

Image Analysis:
Robert Burghardt,
rburghardt@cvm.tamu.edu

Protein Technologies:
Larry Dangott, ljdangott@tamu.edu

Transgenics:
James Martin, jmartin@ibt.tamushsc.edu

Highlight Editor

Alan Parrish,
parrish@medicine.tamu.edu

Center for Environmental and Rural Health
Texas A&M University
4455 TAMU
College Station, TX 77843-4455

Address Correction Requested

This publication is made possible by grant P30-ES09106 from the National Institute of Environmental Health Sciences.