

**SPEAKERS**

**CARL E. GULBRANDSEN, PH.D., J.D.**  
Managing Director, Wisconsin Alumni Research Foundation

**JUDITH KIMBLE, PH.D.**  
Vilas Professor, Department of Medical Biochemistry and Medical Genetics, University of Wisconsin-Madison, and Investigator, Howard Hughes Medical Institute

**WILLIAM LINTON**  
Chairman and CEO, Promega Corporation

**SEAN J. MORRISON, PH.D.**  
Investigator, Howard Hughes Medical Institute, University of Michigan Medical School

**KENNETH D. POSS, PH.D.**  
Assistant Professor, Cell Biology, Duke University

**JANET ROSSANT, PH.D.**  
Professor, Medical Genetics and Microbiology, Obstetrics and Gynecology, University of Toronto; Chief of Research, The Hospital for Sick Children

**ALEJANDRO SÁNCHEZ ALVARADO, PH.D.**  
Investigator, Howard Hughes Medical Institute and Professor, Neurobiology and Anatomy, University of Utah

**ALLAN C. SPRADLING, PH.D.**  
Staff Member, Department of Embryology, Carnegie Institution of Washington; Investigator, Howard Hughes Medical Institute

**CLIVE N. SVENDSEN, PH.D.**  
Professor, Anatomy and Neurology, UW-Madison

**JAMES A. THOMSON, PH.D.**  
Professor, Department of Anatomy, UW-Madison

**RICHARD YOUNG, PH.D.**  
Member, Whitehead Institute; Professor of Biology, MIT

*3rd Annual  
Wisconsin Stem Cell Symposium:*  
**Conserved Mechanisms  
of Stem Cell Control  
and Regeneration**



April 16, 2008  
BioPharmaceutical Technology Center  
Madison, Wisconsin

**OVERVIEW**

Coordinated by the UW Stem Cell and Regenerative Medicine Center and the BioPharmaceutical Technology Center Institute (BTCI), this symposium brings together world leaders in the area of stem cell regulation and tissue regeneration. The focus is on basic molecular mechanisms that are broadly conserved across species and likely to be of clinical significance.

**HIGHLIGHTED ISSUES ARE**

- Chromatin and transcriptional stem cell controls
- Stem cell controls in the embryo
- Stem cells and cancer
- Molecular controls of regeneration

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**SCHEDULE OF EVENTS**

8:00am	Registration and Continental Breakfast
8:45am	<b>Welcome</b> William Linton Carl E. Gulbrandsen Clive N. Svendsen (Moderator)
9:00am	Allan C. Spradling: <b>Regulation of Stems Cells by Chromatin and Competition</b>
9:40am	Judith Kimble: <b>Molecular Controls of Germline Stem Cells in C. elegans</b>
10:20-10:40am	BREAK
10:40am	Janet Rossant: <b>Stem Cells From the Mammalian Blastocyst – Not All Stem Cells Are Alike</b>
11:20am	Richard Young: <b>Transcriptional Regulatory Circuitry of Human Embryonic Stem Cells</b>
Noon-1:00pm	LUNCH
1:00pm	Alejandro Sanchez Alvarado: <b>Stem Cells and Regeneration in the Planarian Schmidtea mediterranea</b>
1:40pm	Kenneth D. Poss: <b>Mechanisms Guiding Organ Regeneration in Zebrafish</b>
2:20-2:40pm	BREAK
2:40pm	Sean J. Morrison: <b>Stem Cells and Cancer</b>
3:20pm	James A. Thomson: <b>Exiting the Pluripotent State and Back Again</b>
4:00-5:00pm	RECEPTION Reception Sponsor: Quarles & Brady, LLP

## ABSTRACTS (IN ORDER OF PRESENTATION)

### Regulation of stem cells by chromatin and competition

Todd Nystul, Michael Busczak, Lucy Morris, Don Fox and Allan Spradling, HHMI/Embryology, Carnegie Institution, Baltimore MD 21218

Adult stem cells hold a special status as relatively undifferentiated, long-term tissue progenitors that can undergo asymmetric, self-renewing divisions. We have identified a novel *Drosophila* gene, *scrumpy*, that encodes an H2B ubiquitin-specific protease. *Scrumpy* mutant animals prematurely lose many kinds of stem cells, suggesting that H2B deubiquitination is a widespread mechanism for suppressing premature stem cell differentiation. Although stem cells maintain tissue structure over the adult lifetime, many individual *Drosophila* stem cells are regularly replaced within their niches by the daughters of neighboring stem cells. We studied long distance replacement using the follicle stem cells (FSCs), which are located laterally in exactly two single-cell niches on opposite sides of the germarium. FSC daughters frequently migrate laterally across the width of the germarium where they target the opposite FSC niche. Cross-migrating daughters usually fail to take up niche residence, but instead differentiate and contribute to the follicular epithelium. About 5% of the time, however, they displace the resident FSC, remain in the niche and function as active stem cells. Stem cell competition may be a common and selectively advantageous adaptation that reduces the chance that deleterious mutations will be maintained in stem cells and the tissues cells they support. Our studies also show that mutations can arise that cause mutant stem cells to preferentially replace wild type stem cells. These "hyper-replacer" mutations may be able to spread from one niche to another until they comprise a substantial portion of tissue and might represent an important new class of precancerous lesion.

Nystul, T. and Spradling, A.C. (2007). An epithelial niche in the *Drosophila* ovary undergoes long range stem cell replacement. *Cell Stem Cell* 1, 277-285.

### Molecular controls of *C. elegans* germline stem cells: Wnt signaling, Notch signaling and on RNAi/MAPK regulatory network

Judith Kimble, Department of Biochemistry, University of Wisconsin-Madison and HHMI, Madison, WI 53706

The nematode *C. elegans* has proven to be a premier model for discovery of fundamental regulatory mechanisms that are used broadly throughout the animal kingdom, including humans. Well known examples include regulators of cell death and RNAi. Stem cell controls are no exception. The concept of a "stem cell niche" was originally derived from studies of vertebrate blood stem cells, but the first cellular candidate for a stem cell niche was the *C. elegans* "distal tip cell". The distal tip cell (DTC) is a single mesenchymal cell that is both necessary and sufficient for maintenance of germline stem cells. The specification of DTCs relies on Wnt signaling during early development, and the maintenance of stem cells by DTCs relies on Notch signaling, both during developmental proliferation and adult homeostasis. The DTC expresses the LAG-2 DSL ligand, and germline stem cells (GSC) express the GLP-1 Notch receptor and transcription factors dedicated to the Notch pathway. When Notch signaling is eliminated, GSCs are lost; when Notch signaling is unregulated, GSCs form a germline tumor. We are just beginning to learn how Notch signaling controls stem cell maintenance. Indeed, Notch signaling promotes the transcriptional activation of two MAPK regulators. One such regulator is PBF-2, a PUF RNA-binding protein that represses MAPK mRNA; the other is LLP-1, a homolog of MKP/DSP dual specificity phosphatases that inhibit MAPK activity. In vertebrates, Notch signaling has been implicated in the control of neural and hematopoietic

stem cells, and MAPK has been implicated in controls of both growth and differentiation. Our analysis of the regulatory network controlling *C. elegans* GSCs is therefore likely to have important parallels for stem cell controls broadly in the animal kingdom.

### Stem cells from the mammalian blastocyst- not all stem cells are alike

Janet Rossant, SickKids Research Institute and the Department of Molecular Genetics, University of Toronto

Three types of permanent cell lines can be derived from the mouse blastocyst embryonic stem (ES) cells, trophoblast stem (TS) cells and extraembryonic endoderm (XEN) cells. All three express markers and show properties in dimers consistent with their origin from the epiblast, trophocytoderm and primitive endoderm lineages of the blastocyst respectively. Key lineage-specific transcription factors, such as Oct4/Sox2, Cdx2 and GATA6/Sox7 determine their fate. Altered expression of these factors can reprogram ES cells to TS or XEN cells. This direct parallel between lineage specification in the embryo and stem cell behavior *in vitro* allows experimental cross-talk between cell culture and embryonic development to provide new insights into stem cell fate. To date, however, it has not proven possible to derive all three permanent progenitor cell lines from human blastocysts. Human ES cells seem to be less lineage-restricted than mouse ES cells, producing some trophoblast and extraembryonic endoderm upon differentiation *in vitro*. We have developed a system to conditionally express lineage-specific transcription factors in human ES cells and show that human ES cells respond differently to mouse ES cells, producing new progenitor cell lines with properties of postimplantation germ layers rather than extraembryonic cell types. These studies and other published reports lead us to propose that there may be more than one stable pluripotent phenotype that can be derived from early embryos or germ cells. Understanding the lineage origin of stem cells is important in order to understand the starting point for driving their differentiation into cell types of therapeutic importance.

### Transcriptional Regulatory Circuitry of Human Embryonic Stem Cells

Richard Young, Whitehead Institute and MIT

The capacity of embryonic stem cells to self-renew and to give rise to virtually all somatic lineages holds much promise for human regenerative medicine. Recent studies have shown that somatic cells can be reprogrammed into an embryonic stem cell-like state. We are mapping the regulatory circuitry of these cells by investigating how transcription factors, chromatin regulators, small RNAs and signaling pathways control the gene expression programs responsible for self-renewal and pluripotency. We are also investigating how genome expression is reprogrammed to produce new cell states. New insights into global control mechanisms will be discussed.

### Stem Cells and Regeneration in the Planarian Schmidtea mediterranea

Alejandro Sanchez Alvarado, Howard Hughes Medical Institute, Department of Neurobiology and Anatomy, University of Utah School of Medicine

The problem of regeneration is fundamentally a problem of tissue homeostasis that involves either the replacement of cells due to normal "wear and tear" (cell turnover), or the replacement of cells after injury. This is particularly significant for organisms possessing relatively long life spans, in which maintenance of all body parts and their functional integration is required for many years in order for the individual to thrive. Replacement of differentiated cells, therefore, is a major challenge all multicellular organisms must face. Humans, for example, with an average

life span of 80 years must replace billions of cells lost to cell turnover every day. Despite the importance of tissue homeostatic processes to human biology and health, relatively little is known about how adult tissue homeostasis is controlled. Gaining mechanistic insight on these problems requires the identification of a model organism in which these issues can be easily dissected and rapidly understood. Key molecular insights can be obtained by studying simpler animals since cellular differentiation events are known to be ancient evolutionary inventions and tissue replacement is broadly distributed among multicellular life forms. Planarians provide a unique and experimentally tractable system for studying such homeostatic, regenerative processes: all tissues are regulated in the adult, and tissue turnover is robust and rapid (as little as 7-10 days). Here, I will discuss how the study of a simple metazoan, the planarian Schmidtea mediterranea, is beginning to shed light on the way adult animals regulate tissue homeostasis and the replacement of body parts lost to injury.

### Mechanisms guiding organ regeneration in zebrafish

Kenneth D. Poss, Assistant Professor, Cell Biology, Duke University

Certain non-mammalian vertebrates like urodele amphibians and teleost fish regenerate complex tissues much more effectively than mammals, creating tantalizing examples of successful organ regeneration. Zebrafish are extraordinarily regenerative, equipped to renew amputated fins, injured retinae, a severed spinal cord, and lost cardiac muscle; plus, they are amenable to both forward and reverse genetic approaches. Current goals in the field are to uncover the responsible cellular mechanisms, and to develop and use tools for high-resolution interrogation of regenerative events at the molecular level. Zebrafish heart regeneration is particularly interesting, since the mammalian heart shows little or no regeneration after injury. In our analysis of zebrafish heart regeneration, we have found evidence that undifferentiated progenitor cells help build new muscle after mechanical injury, and that this activity is facilitated by dynamic responses of the epicardial cell layer surrounding the heart. We are continuing to pursue the cellular origin of regenerated muscle, and to identify molecules synthesized in the epicardium and other cardiac cell types that influence the success of regeneration. Through these investigations, new insights into heart regeneration will be revealed that have the potential to impact regenerative medicine.

### The loss of Nf1 transiently promotes self-renewal but not tumorigenesis by neural crest stem cells

Nancy M. Joseph, Jack T. Mosher, and Sean J. Morrison, Center for Stem Cell Biology, Howard Hughes Medical Institute, and Life Sciences Institute, University of Michigan, Ann Arbor, MI, 48109-2216

Cancers are often proposed to arise from stem cells that have been transformed by mutations that inappropriately activate self-renewal mechanisms. In the peripheral nervous system (PNS), a congenital disorder called neurofibromatosis type 1 is caused by the loss of the neurofibromin (NF1) tumor suppressor, leading to the formation of PNS tumors including neurofibromas and malignant peripheral nerve sheath tumors (MPNSTs). A long-standing question has been whether these tumors arise from neural crest stem cells (NCSCs) or differentiated glia. Gemline or conditional *Nf1* deficiency caused a transient increase in NCSC frequency and self-renewal in most regions of the fetal PNS. However, *Nf1* deficient NCSCs did not persist postnatally in regions of the PNS that developed tumors, and could not form tumors upon transplantation into adult nerves. Adult *Pf1a-Cre<sup>+</sup>Nf1<sup>fl/fl</sup>* mice developed neurofibromas, and *Nf1<sup>fl/fl</sup>;Trk4a/Tr1<sup>fl/fl</sup>* and *Nf1<sup>fl/fl</sup>;53<sup>fl/fl</sup>* mice developed MPNSTs, but NCSCs did not persist postnatally in affected locations in these mice. Instead, MPNSTs and plexiform neurofibromas appeared to arise from differentiated glia that began proliferating inappropriately postnatally or during adulthood. Cancer and benign tumors in the PNS can therefore arise from differentiated glia.

## 3rd Annual Wisconsin Stem Cell Symposium:

# Conserved Mechanisms of Stem Cell Control and Regeneration

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