OEB 200: The Evolution of Stem Cells and Regeneration
Spring 2018
Wednesdays 1-3pm
MCZ 100

Instructor: Mansi Srivastava (mansi@oeb.harvard.edu)

Office Hours: By appointment (NWL 219.20)

Description: Among the many unique challenges that multicellular organisms face is how they deal with the death (or loss) of a part of the organism. Should the organism heal and continue life without the missing part, or should it regrow that part? The vast majority of animal (and plant) lineages have species that will replace the missing portion, *i.e.*, they are capable of regeneration. Despite the phylogenetically widespread nature of regeneration, very little is known about any universal cellular, molecular, and genetic principles, if any, which control this process. In pursuit of these principles, the course will delve into the literature from a diverse range of species. We will focus on stem cells, which enable the production of new cells that reconstitute the missing tissues. We will address what it means to be a stem cell and explore how molecular studies of adult stem cells in a variety of species, ranging from jellyfish to humans, are revealing essential and highly evolutionarily conserved molecular mechanisms for stem cells. We will compare the features of adult stem cells to those of other multipotent cells, such as early embryonic cells and consider how different species maintain pluripotent stem cells versus those with restricted potential, *i.e.*, lineage-restricted stem cells. These ideas will be exlored through the critical reading of primary literature, including both classical and very recent papers in stem cell biology. Students will obtain a deep understanding of the main concepts and methods concerning the study of stem cells and will become familiar with comparative approaches as applied to stem cell biology and regeneration.

Course Objectives: Considering that the evolution of stem cells and regeneration is a topic that is currently unfolding, a major objective for this course is to synthesize information from diverse species and approaches to build a model for how these biological phenomena evolved. In addition to mastering an understanding of classical and current methods for the study of stem cells and regeneration, students will learn how to frame questions in a comparative framework. Students will also learn to critically analyze primary research literature, to effectively communicate their questions and interpretations, and to engage their peers in discussion.

Course Format and Requirements: We will meet once a week to discuss two papers. The discussions will be facilitated by the instructor and two students, who will provide relevant background to frame the discussion.

Leading a Discussion
Each student will sign up to lead a discussion for one meeting (or two, depending on the number of students in the class). The papers listed in the syllabus are provisional; the student-facilitator will meet with the instructor to finalize the paper selection and to plan the discussion. The student will provide relevant background to place the papers in context and facilitate discussion of the paper, including addressing the questions submitted by other students. Please consider using a projector **only** to show movies or other images that cannot be rendered via chalk in order to maintain an atmosphere conducive to discussion.

Paper Discussion Questions
To facilitate discussion, each student will submit to the instructor and to the student in charge of discussion for that week three questions about the papers. These questions should be emailed before **noon** on the day before class (Tuesday). The questions may be about the data, experimental design and methods, the authors' interpretation of the results, or about how the papers relate to other topics discussed in the class.

Short Presentation
During Weeks 11 and 12, each student will pick a paper not yet covered in class to expand our knowledge of mechanisms for regeneration to include more pathways or more species. Students will give a 15-20 minute presentation (chalk talk or PowerPoint/Keynote) about their selection to the class.

Short Final Paper
On the last day of class (Week 13), we will together build a model that compiles hypotheses about the evolution of regeneration and stem cells. Students will write a short (2 single-spaced pages) evaluation of this model detailing an analysis of which aspects of the model are well-supported by the data and which type of new data are needed to validate the model.

Grading: Grading will be based on regular participation in weekly meetings as well as on the assignments. Attendance at all meetings is very important. If absence is unavoidable, the instructor should be notified ahead of time and a make-up assignment will be provided.

Participation in weekly discussion: 25%
Leading a discussion: 30%
Providing questions for weekly discussion: 15%
Short presentation: 15%
Short paper: 15%

Academic Integrity: All written work submitted to the course must be the student's own. Students may discuss work with others, but should write everything in their own words. Students may not copy writings without proper citations.

Academic Honesty: Starting the beginning of the Fall 2015/2016 semester, the Honor Code will be in effect for the academic community of Harvard College.

Members of the Harvard College community commit themselves to producing academic work of integrity - that is, work that adheres to the scholarly and intellectual standards of accurate attribution of sources, appropriate collection and use of data, and transparent acknowledgement of the contribution of others to their ideas, discoveries, interpretations, and conclusions. Cheating on exams, problem sets, plagiarizing, or misrepresenting the ideas or language of someone else as one's own, falsifying data, or any other instance of academic dishonesty violates the standards of our community, as well as the standards of the wider world of learning and affairs.

For resources and guidelines please see: http://honor.fas.harvard.edu

Accommodations for Students with Disabilities: Students needing academic adjustments or accommodations because of a documented disability must present Mansi Srivastava with a letter from the Accessible Education Office (AEO) and speak with her by the end of the second

week of the term. Failure to do so may result in the course's inability to respond in a timely manner. All discussions will remain confidential, although faculty may contact AEO to discuss appropriate implementation.

Course Schedule:

Module I – Stem Cell Basics
Module II – Cellular and Molecular Pathways for Regeneration
Module III – Synthesis

Week, Topic & Date	Description & Papers
1 **Module I** Organization and Intro: Stem Cells and Regeneration	The objective of this first class will be for us to get acquainted with each other, and with the topics of the various sessions and how they relate to each other and the central theme of stem cell biology and regeneration. We will have a discussion about how we define stem cells and why we associate them with regeneration. We will also discuss what we currently know about stem cells and regeneration from various species, in their phylogenetic contexts.
January 24, 2018	
2 **Module I** Discovery of Stem Cells	We will read a seminal paper about the hematopoetic stem cell system. This paper represents the beginnings of modern day stem cell research. The second paper, published 37 years later, will detail the discovering of a major progeny of the hematopoetic stem cell, the common myeloid progenitor. The objective in reading these papers will be to focus on the methods that ultimately led to the in-depth understanding we now have about hematopoietic stem cells, the most well-studied stem cell system.
January 31, 2018	1. A.J. Becker, E.A. McCulloch, J.E. Till. 1963. Cytological demonstration of the clonal nature of spleen colonies derived from transplanted mouse marrow cells. *Nature.* 197(4866):452-454. 2. A clonogenic common myeloid progenitor that gives rise to all myeloid lineages. 2000. Akashi K, Traver D, Miyamoto T, Weissman IL. *Nature*. 404(6774):193-7.

3 **Module I** Stem cell potency and dynamics	How do you find an adult stem cell? And how do you then find out what cell types it makes and doesn't make? This week we will read papers about two different adult stem cell systems and learn which techniques and strategies researchers have used to study stem cells and their potency. The first paper describes the identification of the intestinal stem cell. The second paper focuses on understanding proliferation dynamics to identify exactly which cells contribute to skin regeneration.
February 7, 2018	1. Barker N, van Es JH, Kuipers J, Kujala P, van den Born M, Cozijnsen M, Haegebarth A, Korving J, Beghtel H, Peters P, Clevers H (2007) Indentification of stem cells in small intestine and colon by marker gene Lgr5. *Nature(449) 1003-8* 2. Mascré, G., Dekoninck, S., Drogat, B., Youssef, K. K., Brohée, S., Sotiropoulou, P. A., Simons, B. D. and Blanpain, C. (2012). Distinct contribution of stem and progenitor cells to epidermal maintenance. Nature 489, 257-262.

4 **Module I** The niche	Adult stem cells in animals are tightly controlled by essential cues originating from neighboring cells and environment, often referred to as the "niche". The first paper is an elegant study that utilizes intravital microscopy to study the control of stem cell fate by the niche in mammalian hair follicles. The second paper presents a very different phenomenon in plants, where stem cell niches appear not to be needed for regeneration.
February 14, 2018	1. Panteleimon Rompolas ; Kailin R. Mesa ; Valentina Greco. (2013) Spatial organization within a niche as a determinant of stem-cell fate. Nature. 502(7472):513. 2. Sena G, Wang X, Liu HY, Hofhuis H, Birnbaum KD. (2009) Organ regeneration does not require a functional stem cell niche in plants. Nature. 457(7233):1150-3.

5 **Module I** Mechanisms for Pluripotency and Differentiation	Stem cells need to self-renew and stay in a pluripotent state, whereas their progeny have to be able to differentiate. Much research has been devoted to the question of how stem cells maintain their potency. *ex vivo* studies of cultured embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) have revealed many essential mechanisms for pluripotency

	and differentiation. The first paper is the report of the famous "Yamanaka" factors, which transform differentiated cells into pluripotent cells. The second paper looks at chromatin regulation during differentiation by focusing on the role of FoxD3 in the formation of epiblast cells from embryonic stem cells.
February 21, 2018	1. Takahashi, K. and Yamanaka, S. 2006. Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors. Cell. 126:663–676. 2. Krishnakumar, Raga, Amy F. Chen, Marisol G. Pantovich, Muhammad Danial, Ronald J. Parchem, Patricia A. Labosky, and Robert Blelloch. 2016. FOXD3 Regulates Pluripotent Stem Cell Potential by Simultaneously Initiating and Repressing Enhancer Activity. *Cell Stem Cell* 18 (1): 104–17.

6 **Module II** Cells for vertebrate regeneration	Humans have limited capacity to replace missing structures, but other vertebrates, such as fish and salamanders, are able to regenerate limbs, tail fins, and larval hearts. We will read two papers that explore which stem cell populations respond to injury in vertebrate regeneration. The axolotl salamander is famous for its ability to regenerate limbs. For many years it was thought that this regenerative response was due to dedifferentiation of cells at the amputation site to a pluripotent state. These newly acquired pluripotent cells would then be the source of all new tissues in the regenerating limb. The first paper revisits this model with modern-day techniques and finds that reality is more complicated. The second paper describes stem cell function in the only similar process known in mammals: digit tip regeneration.
February 28, 2018	1. Kragl M, Knapp D, Nacu E, Khattak S, Maden M, Epperlein HH, Tanaka EM. 2009. Cells keep a memory of their tissue origin during axolotl limb regeneration. *Nature*. 460(7251):60-5. 2. Lehoczky JA, Robert B, Tabin CJ (2011) Mouse digit tip regeneration is mediated by fate-restricted progenitor cells. *PNAS.* 108:20609-20614.

7 **Module II** Cells for invertebrate regeneration	Planarians are well-known for their storied ability to regenerate any missing tissue. Thomas Hunt Morgan studied these flatworms before moving on to fruit flies and inventing modern genetics. We will read a paper that demonstrates the pluripotent capacities of a single planarian stem cell. We will use this study as a framework for discussing the burden of evidence needed to truly establish pluripotency in any new system we discuss. The second paper will introduce a very different stem cell system, one found in another invertebrate species, the colonial sea squirt *Botryllus*. We will discuss how the two stem cell systems compare to each other and to stem cell systems in vertebrate regeneration.
March 7, 2018	1. Wagner DE, Wang IE, Reddien PW. 2011. Clonogenic neoblasts are pluripotent adult stem cells that underlie planarian regeneration. *Science*. 332(6031):811-6. 2. Rinkevich Y, Voskoboynik A, Rosner A, Rabinowitz C, Paz G, Oren M, Douek J, Alfassi G, Moiseeva E, Ishizuka KJ, Palmeri KJ, Weissman IL, Rinkevich B. 2013. Repeated, long-term cycling of putative stem cells between niches in a basal chordate. *Dev Cell.* 24(1):76-88.

8 **Module II** Are pluripotent stem cells ancestral?	During the previous discussion, we read two papers pointing to the presence of pluripotent adult stem cells in invertebrates. We will dig deeper into planarian stem cells with the first paper, which was the first study to analyze them at the single-cell level. We will then compare the inferences from this paper to those in the second paper, which suggests the presence of lineage-restricted stem cells in crustacean limb regeneration.
March 21, 2018	1. van Wolfswinkel JC, Wagner DE, Reddien PW. 2014. Single-cell analysis reveals functionally distinct classes within the planarian stem cell compartment. Cell Stem Cell. 15(3):326-39. 2. Konstantinides N, Averof M. 2014. A common cellular basis for muscle regeneration in arthropods and vertebrates. Science. 343(6172):788-91.

9 **Module II**	Some animal species lack adult stem cells entirely. The only cells capable of producing other cell types in these animals

Germ cells – the ultimate immortal cells	are germ cells, which produce the the oocytes and sperm that make a zygote, which ultimately gives rise to all cells of the adult. This week we will read about the germ cell system in the fruit fly *Drosophila*, focusing on Piwi, a protein you have already encountered in other stem cell systems. The first paper will explore the intriguing cellular structures that Piwi proteins are found in, and the second provides one mechanism through with Piwi might operate to maintain stem cells.
March 28, 2018	1. Trcek T, Grosch M, York A, Shroff H, Lionnet T, Lehmann R. 2015. Drosophila germ granules are structured and contain homotypic mRNA clusters. Nat Commun. 6:7962. 2. Peng, Jamy C., Anton Valouev, Na Liu, and Haifan Lin. 2016. Piwi Maintains Germline Stem Cells and Oogenesis in Drosophila through Negative Regulation of Polycomb Group Proteins. *Nature Genetics* 48 (3): 283–91.

10 **Module II** Immortal animals, immortal cells	Some animals, particularly those with pluripotent adult stem cells, appear to have unlimited life spans. The first paper focuses on one such system, Hydra, to understand the role of a Fox family transcription factor, FoxO, in controlling stem cells. FoxO has also been shown to play a role in aging in other animals. Most animals have limited lifespans; in our bodies, aging is accompanied by a decline of stem cell populations. The second paper seeks to understand mechanisms that control aging of adult stem cells in vertebrates. The objective of this class is to understand how mechanisms for aging relate to stem cells and regeneration.
April 4, 2018	1. Boehm AM, Khalturin K, Anton-Erxleben F, Hemmrich G, Klostermeier UC, Lopez-Quintero JA, Oberg HH, Puchert M, Rosenstiel P, Wittlieb J, Bosch TC. FoxO is a critical regulator of stem cell maintenance in immortal *Hydra*. 2012. Proc Natl Acad Sci U S A. 109(48):19697-702. 2. I. M. Conboy, M. J. Conboy, A. J. Wagers, E. R. Girma, I. L. Weissman, T. A. Rando. Rejuvenation of aged progenitor cells by exposure to a young systemic environment. *Nature* 433, 760–764 (2005).

11 **Module III** Student presentations	Students will present papers to add molecular pathways and phylogenetic breadth.
April 11, 2018	

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April 18, 2018	

13 **Module III** Build a model	We will assemble a model for the evolution of stem cells and regeneration by annotating a phylogenetic tree with pathways that we have learned about over the course of the semester.
April 25, 2018	