

# Genesis of Gametogenesis

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Thesis submitted in partial fulfillment of a Bachelor's of Arts

with Honors in Science and Society

Brown University

April 29<sup>th</sup> 2015

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“In our view biology as a whole, and the origin of meiotic sex in particular, is ready for a paradigm shift” (Margulis, *Origins of Sex*)

## Table of Contents

Acknowledgments.....	4
Abstract.....	5
Introduction.....	6
Methods.....	9
Preface.....	16
Chapter 1: Historical Differentiation of the Germline.....	30
Chapter 2: Deep Homology and the Reproductive Genome.....	42
Chapter 3: Enter the Gametes: Relating the Mammalian and Plant Germlines.....	62
Chapter 4: Transgenic Futures of the Germline and Stem Cells.....	85
Conclusion .....	103
Works Cited .....	107

## Acknowledgements

Dr. Sherine Hamdy's encouragement and influence are a foundational part of my undergraduate science and technology studies experience. I benefitted greatly from the opportunity to try on the identities of laboratory scientist and ethnographer simultaneously during the course of this thesis and relied on her persistent encouragement when my confidence faltered. Thanks for your commitment to participate in academia as an STS scholar and continual support of likeminded undergraduates.

Thanks to the Pollen Genetics Lab for extending a warm welcome and humoring my zany presence over the past year. I'm especially grateful to Jen Forcina for direct oversight and assistance in much of the day-to-day lab work, in addition to review of drafts. Thanks to Drs. Mark Johnson and Alison DeLong for being especially supportive of undergraduate research and for providing enthusiastic encouragement even if I didn't quite understand what I was getting myself into. I'd also like to extend my gratitude to Dr. Alex Leydon and Austin Draycott for answering several basic questions that they may or may not have had the time but expended the energy and maintained a good sense of humor throughout.

To Dr. Richard Freiman: thanks for two semesters of engaged undergraduate teaching and support both in the classroom and in lab. I'm looking forward to another year at Ship St. and hope to see you often. Also thanks to all of the fabulous "TeamT," you are the center of my "lab life" and some of my closest friends here at Brown. Growing up with the lab continues to be a rewarding experience. The mentorship of both Dr. Boekelheide and Maggie Vantangoli has been particularly formative over the past three years. Thank you!

Finally, thanks to family and undergraduate friends for copy-editing drafts and dealing with my bad humor when I was under-slept or frustrated with lab work. The show's over now, but I couldn't have done it without you.

## Abstract



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The definition of germline stem cell (GSC), an entity defined by some biologists as giving rise to egg or sperm in sexually reproductive organisms, is in flux. Past paradigms of reproductive biology relied on anthropocentric experiments to locate meaning in both “gamete” and “stem cell,” almost exclusively within metazoan (animal) bodies (Solana). As a result of conceptual “instabilities” in this definition, the identity of GSCs in 21<sup>st</sup> century molecular biology is multiple, evolving, and generative. The source of these instabilities includes a reconceptualization of scale, a redefinition of biological sex and reproduction, and the emergence of speculative biotechnologies that produce novel forms of life and biocapital. Study of GSCs actively determines the process of biological reproduction and the limits of organic bodies, negotiating the relationship between varied forms of life (Waldby and Squier). In this report, the process and products of science involving GSCs are explored. Using primarily ethnographic methods, data was collected through participant-observation during the production of original laboratory research in three Brown University labs. The following historically informed, ethnographic and scientific account of GSCs is enhanced through development of and participation in a pilot study of one mammalian GSC related gene in alternative model systems. As a collaborative endeavor, direct engagement with laboratory science provided context for material understanding and reflection between multiple scientists and the “ethnographer-participant.” Historically understudied in Science and Technology Studies (STS), research involving organisms of no immediate value to biomedicine are shown to have unrecognized and disproportionate transformative power in biological knowledge production.

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## Introduction

*“...The image of a unified natural science still informs the social sciences and contributes to their dominant theoretical and methodological orientation... interpretations of science all tend to assume science is a unitary enterprise to which epistemic labels can be applied across the board. The enterprise, however, has a geography of its own. In fact it is not one enterprise but many, a whole landscape- or market- of independent epistemic monopolies producing vastly different products.”*  
(Cetina)

Germline stem cells give rise to egg and sperm through the process of gametogenesis in sexually reproductive, multicellular organisms. More accurately, the term “germline stem cell” is a tool employed by biologists to understand the persistent separation of somatic cells from gametes, cells assumed to be uniquely capable of meiosis, syngamy, and eventual embryogenesis post-fertilization. As a result of conceptual instabilities, the working definition of germline stem cell (GSC) remains multiple, evolving, and generative in 21<sup>st</sup> century molecular biology. The sources of instability in these defining paradigms include a reconceptualization of scale, a redefinition of biological sex and reproduction, and the emergence of speculative biotechnologies that produce novel forms of life and biocapital. Further, GSC’s hybrid and possibly competing identity as both long-lived, proliferative stem cell (SC) and germline, or gamete producing unipotent cell, entitle them to a central, transformative role in the production of biological science. The perpetuation of a static definition of GSC relies on anthropocentric experiments that locate meaning in both “gamete” and “stem cell,” almost exclusively within metazoan (animal) bodies. Such accounts have historically been used to naturalize “social conventions of gender” and hierarchies of model organisms (E. Martin, “The Egg and the Sperm”). As investigators increasingly

study GSCs in model systems that defy 20<sup>th</sup> century research paradigms, their laboratory investigations catalyze a redefinition of formerly uncontested language and lead to conceptual instabilities in the static definition of GSC.

Stem cells and gametes remain foundational entities in the development of molecular biology over the past century, and serve as material and theoretical engines of production. The manipulation, alteration, and control of these cell types is reflective of an epistemic culture (Cetina)- that is, the unarticulated conditions of knowledge production- organized around harnessing the means of biological (re)production (Haraway, “A Manifesto for Cyborgs”). As material entities at the nexus of this epistemic culture, the boundary maintenance required to define GSCs, SCs and gametes is locally determined, varying widely with laboratory context (Gieryn). Emergent biological research utilizing non-mammalian model systems and *in vitro* cell culture occurs at the margins of cellular life and exemplifies how different “epistemic monopolies” are producing increasingly variable “products” (Cetina) in the amalgamated and highly speculative disciplines of stem cell and reproductive biology.

Two striking examples of how and where this difference emerges include the study of non-classical model organisms (organisms other than fruit fly or mouse, for example) and *in vitro* stem cell engineering, in which cell identity is often nebulous and transitional but inherently generative. To further illustrate and explore the multiplicity of identities ascribed to GSCs, this report evaluates and encompasses the production of original laboratory research in three Brown university labs. Each lab analyzed studies gametogenesis or its products, egg and sperm. Origin stories of SCs, GSCs, and gametes emerge in context with laboratory practice through diverse verbal and visual sign

systems. Their origins reflect the volatile construction of difference between organism and cell colony, gamete and germline, and natural from scientific means of reproduction. Further, scientific conventions of “stemness,” the property enabling stem cells to maintain proliferative capacity and give rise to multiple differentiated “daughter cells” (Morrison and Spradling), is found to be context dependent. GSCs must ultimately be understood as transformative scientific tools because they serve a pivotal role in defying and redefining cultural narratives of gender, reproduction, and limits of cellular life.



## Methods

The data collected for this report combine ethnographic study of laboratory science, quantitative and qualitative data relating phenotypic, developmental and genetic observations collected as a laboratory technician, and historical analysis of select biological publications. The “imprecise” ethnographic methods of “‘hanging out,’ ‘writing field notes,’ and ‘following things around,’” described by Franklin in her treatise on the “double reproductive value” of stem cells provide unique insight into what Latour describes as “science in action.” I found the organic, conversational nature of such research strategies to be key in comprehensively evaluating the practice of laboratory science. It is within this framework of participatory Science and Technology Studies scholarship, toeing the line between partnering directly with scientists in research and possibly alienating them through pointed anthropological study, that I developed my methods. As my pilot investigations and sense of scientific citizenship progressed, relating an entirely “objective” account of research activities became necessarily impossible. My direct involvement with research and responsibility for directing the progression of the pilot make this embodied account of laboratory science and ethnography more nuanced, complex and aware.

I conducted ethnographic fieldwork in collaboration with three Brown university labs and three undergraduate courses. Each lab investigates some aspect of gametes or gametogenesis in various model systems ranging from *Arabidopsis thaliana* to human cell lines. Interview and participation in three undergraduate courses at Brown University including *Stem Cell Biology*, *Reproductive Biology* and a seminar in Science and

Technology Studies adds to the breadth of data collected, developing my analysis of how scientific paradigms are codified and reproduced for novice scientists. My role within the context of fieldwork ranged from direct engagement with laboratory research as a technician, enrollment in undergraduate courses as student, participation in lab meetings as an employee, and informal interviews as a pupil of both junior and senior scientists. The format of data collected is equally diverse integrating both visual laboratory assays and verbal symbols from laboratory notebooks, recorded interviews, and ethnographic field notes.

Stage one of research seeks to capture what Latour describes as “science in action,” and involves studying the activities of scientific investigators as an ethnographer-participant directly engaged with the laboratory context. During this stage, my use of recorders to later analyze conversations in the laboratory precipitated a change in the candor of discussion and the use of more formalized language. Thus, I made exclusive use of field notes and written documentation when in the fast-paced context of “lab.” This less intrusive method is necessary to accurately capture the ebbs and flows of lab life. The colloquialisms, banter, and humor present in each context are reported here and accurately reflect the lived experience of lab culture. Stage two reflects a distilled vision of laboratory science- informed by initial field work- back to investigators, seeking their interpretation of these observations in participatory feedback loops. I accomplished this through a series of recorded interviews in an open discussion format, capitalizing on the familiarity I gained over the course of previous ethnographic fieldwork and laboratory investigation.

I conducted ethnographic fieldwork and/or laboratory research in the Boekelheide, Freiman, and Johnson labs, each headed by a senior, tenured professor at Brown University. The research conducted in Dr. Kim Boekleheide's lab, known by some students and collaborators as "Team Testis," is organized around the study of human endocrine disruption and toxic insult to the male reproductive system. The breadth of the Boekelheide Lab's research efforts were recently expanded to include the development of a high throughput baseline toxicity-testing platform. With this model, they hope to perfect an *in vitro*, human cell culture system that accurately recapitulates *in vivo* toxicant exposure mechanisms. As a part of the pathobiology and laboratory medicine departments at Brown, Team Testis encounters gametogenesis and GSCs in the context of toxicant induced pathology, predominantly in model systems including mouse, human-rat xenotransplant studies, and human cell lines. Reflective of the field of stem cell biology at large, the ultimate goals are biomedical in scope and execution.

Dr. Richard Freiman and his lab members study transcriptional regulation and its relevance to mammalian disease using a molecular genetic approach. Under the Molecular Biology, Cell Biology and Biochemistry department at Brown, much of the Freiman lab's investigations involve the transcription factor TAF4b, (TATA-binding associated factor 4b), and its role in maintenance of the mammalian GSC population. This gene and its potential homolog in the plant model organism *A. thaliana* are the basis of a collaborative pilot study with the Johnson lab. Their collaboration allowed me a unique platform for observing how scientists negotiate and maintain boundaries between gametes, GSCs, and SCs in a comparative context.

Finally, the work of Dr. Mark Johnson and his students, collectively known as the “Pollen Genetics Lab” (PGL), includes investigation of genetics mechanisms guiding plant fertilization in the model organism *Arabidopsis thaliana* (*Arabidopsis*) and *Solanum lycopersicum* (Tomato). Also under the Molecular Biology, Cell Biology and Biochemistry department at Brown, Dr. Mark Johnson is a colleague in the same cohort as Dr. Freiman, both having joined the department within a year of each other. One graduate student remarked on their collegial relationship, joking: “you’re lucky that they get along, [though] it might actually make things slower at times” (Freiman and Johnson, “TAF4b Update September”). My lab ethnography is primarily based in the PGL, but also included meetings with Drs. Freiman and Johnson and various other junior and senior scientists.

I adapted the format of data collection to fit each unique context. Ethnography conducted in the PGL was documented extensively with field notes, email exchanges, and a wide variety of lab-produced quantitative or qualitative data. The limited meetings in which Drs. Johnson and Freiman and/or their junior and senior researchers were present were often recorded electronically and then transcribed. My ethnographic study of Dr. Kim Boekelheide’s lab did not employ electronic recording devices, but also made extensive use of informal conversation, observation, and note taking. My presence over a two year period in the Boekelheide lab ensured a high degree of familiarity, and thus candid remarks during interactions with scientists. In direct contrast to methods of lab ethnography, electronic recording devices formed the basis of investigations in the classroom context of “Stem Cell Biology,” taught by Dr. Richard Freiman. Throughout the remainder of this report, the identity of principle investigators Mark Johnson, Gary

Wessel, Kim Boekelheide and Richard Freiman are necessarily revealed, while most PhD students, undergraduates, and other senior researchers encountered are not named.

Over the course of a year, recordings collected during the Brown University courses, “Stem Cell Biology” and notes from “Reproductive Biology” provided direct insight into the culture and politics of these fields. As a participant in the Stem Cell Biology classroom context I received permission to use a recording device and to quote Dr. Freiman and anonymous fellow students. Handwritten notes taken during Reproductive Biology allowed me to similarly evaluate classroom discussion. My methods were adapted to reflect the more formalized context in which undergraduate students are expected to, and would benefit as “scientific citizens” from, using formalized scientific language (Cetina). Relying heavily on review of scientific publications, these classroom conversations demonstrated both the inculcation of novice student-scientists and peer review of contemporary (and limited historical) molecular biology. The presentation of various publications by these professors to their students provided insight and exposure to “ready made” science and thus the ongoing inheritance of scientific paradigms. Diverse topics ranging from sexism in science to fraud in stem cell biology were also explored in conversation with professor and students.

In the process of imagining a course of laboratory experiments, it became necessary- as is increasingly necessary in most genetic and molecular biology research- to communicate information through visual symbols and language. Storytelling in the context of the PGL necessitated several drawings and computer animations to communicate processes and products at the level of genetic sequence and macromolecule. The reliance on visual sign systems during the production of science in PGL is reflective

of the greater narrative culture (Cetina) characteristic of molecular biology. Several times throughout the process of lab research Dr. Johnson encouraged me to “think about the figures” (Freiman and Johnson, “TAF4b Update September”) that would make up a hypothetical publication, and use that as a guide for the directions to take my research. At the beginning of each “experimental” chapter I’ve chosen to include Johnson’s original sketches: the hypothetical figures and the experiments they represent. Cetina suggests that this “narrative,” and the formulaic story produced, i.e. the scientific publication, necessarily allows other biologists to empathetically imagine themselves in the investigative process. This experience was reproduced in my laboratory research by Johnson’s encouragement to let the imagined figures of a future publication guide the production of ongoing research. A successful publication was thus necessarily an empathetic one. For this reason all manner of explanatory sketches, draft figures, and computer-generated graphics will be included in the body of my report.

Regarding the format and style of this report: there are five goals, or assurances, made to readers. The first is the avoidance of jargon without adequate explanation or context. I intend for the text to be accessible to academics and curious people of diverse intellectual persuasions. Wherever jargon is referenced, an explanation of relevant theoretical, technical, and historical background will be provided. Second, I hope to provide insight that transcends publication date, producing a narrative that doesn’t dwell on or become static beyond its native time frame. Assurances three and four: given that sections of this report may resemble “scientific narrative,” as Lynn Margulis describes some of her work, every attempt to clearly define my relationship to context (so as to acknowledge personal bias) and make known rhetorical weakness in specific arguments

will be made. This is accomplished in hopes of producing an earnest and honest product that may stimulate empathy and engagement. The final stylistic feature addressed here is the inclusion of “raw” data from all the various primary sources. Whether it is a section of transcribed interview, printout from a laboratory procedure, or sketches created by scientists to communicate technical information visually, all forms of data will necessarily be included in this report. In reading the work of Cetina and Martin, I was grateful for the inclusion of quotations from their laboratory ethnography because it allowed me to come to different conclusions than those reached by the authors. Thus, I hope that readers will engage with the primary source material presented, and that it may stimulate new, different and potentially contradictory lines of thinking.

Additional descriptions of laboratory methods and molecular biology terminology can be found in the referenced material. Often, in text descriptions are cursory to avoid lengthy interjections of procedural and technical jargon that would otherwise distract or muddle rhetoric. The interested reader is encouraged to refer these sources as needed to supplement cursory definitions provided in the body of this report. I do not make the claim that my descriptions of scientific procedures are comprehensive or that all linguistic definitions are precise; on the contrary, many of these terms may be contested and subject to several different contextual meanings and histories.

## Preface



**Figure 1:** View inside the “cold room” used by Pollen Genetics Lab for storing bacterial cultures, fresh petri dishes, and unused *Arabidopsis* seedlings

*“Understudied model systems and novel technologies such as induced pluripotent stem cells (iPSCs) are forcing us to question long-established concepts...such efforts may ultimately help to marshal an age of biological discovery unconstrained by the incrustations of familiarity.” (Sánchez Alvarado and Yamanaka)*



What is the material basis of sexual reproduction? This question underlies the development of biological science from Aristotle to Gurdon and serves as a unifying though increasingly heterogeneous engine of biological knowledge production. This fundamental problem interrogates “the very beginning of life” and is unsatisfactorily answered with the tools of molecular biology, history, genetics or anthropology alone. The manipulation, curation, and control of gametes and other reproductive tissues by humanity is reflective of a pervasive and dynamic epistemic culture (Cetina) structured around harnessing the means of biological (re)production (Haraway, “A Manifesto for Cyborgs”; Helmreich, “Species of Biocapital”). In this frame, “the mouse, the cell, the bacteria and the vector have become production devices in themselves,” (Cetina) lively agents in the biotech industry. One particular cell type privileged by this culture is the germline stem cell, a cell type that is understood to give rise to gametes in sexually reproductive multicellular organisms. By focusing on the fluid definition of “germline” and “stem cell” it is possible to appraise the production and heterogeneous products of reproductive and stem cell biology. This report evaluates how germline stem cells shaped are within the social and material body by forces as diverse as evolution, teratogens, and societal needs. Also addressed here is the question of why GSCs materialize- or fail to – in a given context.

Both of the labs I currently call home study male gametes (sperm) and gametogenesis, and thus provide a crucial vantage point in conducting ethno-science research. Each lab employs vastly different tools in investigations of superficially similar concepts, namely the characterization of healthy sperm production and function. The “comparative optic” provided by research based in multiple laboratory contexts is crucial.

As Cetina notes in *Epistemic Cultures*, the “differences” between scientific practices and disciplines are often “far more tractable than the essential features.” Cetina’s lab-based ethnography straddled the fields of particle physics and molecular biology, fields much more varied than will be addressed in this report. Mark Johnson’s lab, the “Pollen Genetics Lab,” investigates the genetics of pollen, in particular sperm delivery via the pollen tube and gamete fusion. and “Team Testis,” headed by PI Kim Boekleheide, specializes in the toxicology of mammalian testes. Both Boekelheide and Johnson’s research questions are implicitly motivated by perceived risks to gametogenic function—namely global warming and its negative effect on plant fertilization in the case of PGL, and human toxicant exposures and resulting cancer, endocrine disruption, or infertility in Team Testes. The uniquely privileged status of gametes, positioned alternatively as both fragile, latent life at risk or tool for harnessing the means of sexual production, motivates and defines a deluge of biological research across increasingly diverse model organisms and experimental methodologies.

My initial exposure to gametogenesis and GSCs evolved in the context of Kim Boekelheide’s reproductive toxicology lab. Dr. Boekelheide and his students conduct research aimed at elucidating the various mechanisms by which endocrine disrupting toxicants interfere with spermatogenesis and cause other testicular or developmental injuries. From fetal testis development and teratology involving early spermatagonial stem cells, to the development of an *in vitro* system for assessing endocrine disrupting toxicants, work in the Boekelheide lab invites diverse methods and goals. At the most basic level all research is related to testicular pathology and biomedical human health applications. Though germline stem cells are not the sole object of study, their presence

continues to be measured and tracked carefully across the decades, inferred through gene expression data, protein assays, and histology. In the Boekelheide Lab, extensive time and effort goes into the measurement of histological and cellular morphology endpoints extracted from microscope images, an activity most students will colloquially refer to as “scoring.” As a medical doctor and research scientist, Dr. Boekelheide sees with a pathologist’s lens. The epistemic culture of his lab privileges biomedical and therapeutic interpretation of spermatogonial stem cells; reflective of the majority of stem cell research in the 21<sup>st</sup> century. The lab is concerned with risk of cellular dysfunction resulting from toxicant exposure, and frames most questions involving fertility, development, and germline stem cells relative to the disruption of “natural” developmental outcomes.

While immersed in the Boekelheide lab I became especially fascinated by the potential for multi-generational impact via fetal GSC teratogens, including diethylstilbestrol (DES). Prescribed in Europe during the mid 20<sup>th</sup> century as a drug intended to prevent miscarriage, DES was later found to be extremely carcinogenic and manifested in various cancers in the generation exposed in utero (Bell). Many individuals exposed as fetuses developed rare forms of cancers early in their adult years. Human germline stem cells are susceptible to irreversible toxic insult even months before birth. A single genetically damaged germline stem cell or primordial germline stem cell holds the potential to impact several lives across generations of organisms via inheritable epigenetic modifications. This sort of “biographical biology,” (Waldby and Squier) in which future community and hypothetical life trajectories are imagined, epitomizes that risk is embodied by a single, discreet generation. Waldby and Squire ask: can the

“trajectory of human biological life...preserve identity across time?” Xenobiotics insult of the fetal germline stem cells has the potential to change the “temporal trajectory of human life” across generations, and correspondingly Waldby and Squire suggest that stem cell alterations- *in vitro* or *in vivo*- have profound implications for our “ways of being human” (Waldby and Squier).

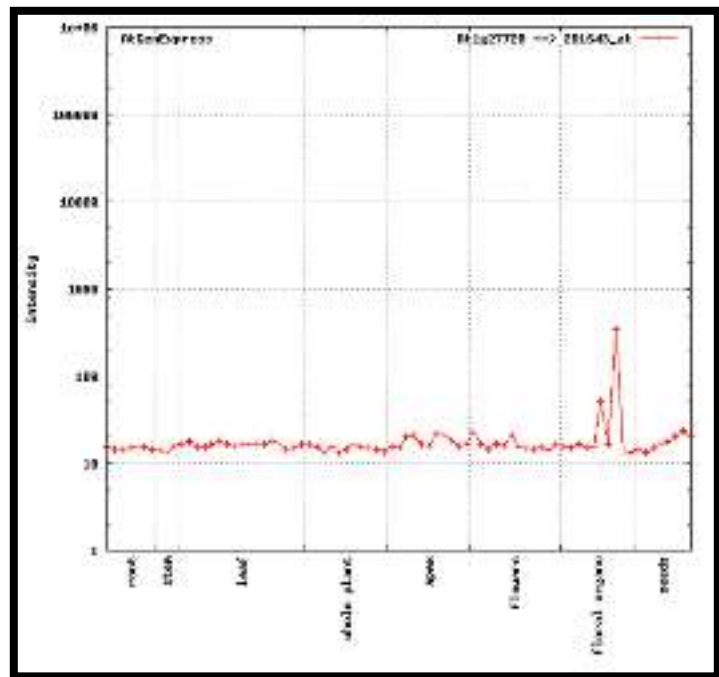
During this period I was concurrently enrolled in Dr. Freiman’s *Cell Biology* course and *Plant Organism*, taught by Drs. Johnson and DeLong. The simultaneous inquiry from a metazoan-centric and plant-based curriculum motivated me to explore the seemingly volatile scientific definitions of “germline stem cell” relative to animal and plant models. How are the evolutionary histories and contemporary developmental realities of gametes defined heterogeneously relative to the multi-cellular body in which they reside?

During one particularly formative *Cell Biology* lecture, Freiman presented his lab’s work involving the gene TAF4b, TATA box binding associated factor 4b, a transcriptional regulator of mammalian GSCs. In 2005 Freiman and his collaborators found that TAF4b “knock out” (genetically deficient) mice are unable to re-designate or maintain the germ line stem cell population, resulting in eventual sterility in male mice and an ovarian aging phenotype in female mice (Falender et al.). Coincidentally, a lecture given around this time by Drs. Johnson and DeLong in *Plant Organism* introduced the *Arabidopsis* mutant, *MSI* (male sterile 1). This mutant plant fails to produce normal male floral organs- stamens and pollen- the rough functional equivalent of testes and sperm found in sexually reproductive animals (Vizcay-Barrena and Wilson). The timing of these two lectures, each introducing a genetic mutation that interrupts germline formation

captured my attention. How did specialists, in particular Boekelheide, Freiman and Johnson, conceive of the material basis of the germline? What basic genetic components of their model systems were understood to overlap, including the gene TAF4b, or *MS-1*, and would the scientific language these biologists used to describe their model systems reflect coherence in the term “germline” and “germline stem cell?”

Though my over-eager novice questioning quickly revealed that there was no direct relation between the *MS-1* gene in *Arabidopsis* (Wilson et al.) and the mouse TAF4-b gene in Freiman’s publication, the lack of functional male gametes in both the *Arabidopsis* and mouse mutants was intriguing, and provided a powerful tool in studying the germline. *MS-1* plants were used heavily at various points in my PGL investigations, from the production of the GFP expressing transgenic plant in experiment three to

analysis of the mutant TAF4b plants phenotype. Thanks to some encouragement from Drs. Johnson and Freiman, a search in the AtGenExpress Visualization Tool (Schmidt, Schmid, and Grossniklaus) revealed that a TAF4b homolog existed in *Arabidopsis*, and was “really germline specific” (Freiman and Johnson, “Winter TAF4b Meeting, PGL and Freiman Labs”). AtGenExpress Visualization



**Figure 2:** *Arabidopsis* TAF4b gene expression data from AtGenExpress Visualization Tool; peaks at right depict pollen specific expression pattern

Tool (AVT) is an open source of gene expression data, and reflects the relative rate of protein accumulation over developmental time.

After learning that *Arabidopsis* possessed a TAF4b homolog, that is, a gene similar in sequence to the mouse TAF4b gene, and further, that TAF4b was expressed in pollen, I began scouring the literature for publications. Finding none, I wondered whether there was an evolutionarily conserved function, “deep homology,” in TAF4b’s regulation of gametogenesis despite the fact that the last common ancestor between extant plant and animal groups was unicellular.

Thus, having developed rapport through undergraduate coursework I suggested that and Drs. Freiman and Johnson embark on a collaborative investigation into the *Arabidopsis* equivalent (homolog) of TAF4b. Both Freiman and Johnson agreed to support a pilot investigation into the TAF4b homolog, employing me as a primary (volunteer) technician in the process and permitting science ethnography throughout the course of lab based research. What follows grew from the collective efforts of these two principle investigators, their students, and myself.

How might the genetics of sexual reproduction provide a comparative optic across discontinuous bodies and modes of biological inquiry? Undergraduate curiosity and relative outsider-status positioned me perfectly as a layperson-ethnographer. In describing fieldwork for “Flexible Bodies,” Emily Martin suggests that the experience of “visceral learning” in a immunological laboratory setting was more enlightening and nuanced than “verbal questions put directly” to scientists (E. Martin, *Flexible Bodies*). Similarly empowered with a relative ignorance, I could ask questions about the production of these complex knowledge systems that may not be conventional

in laboratory culture. Straddled between toxicological, genetic, metazoan, and plant-based investigations of the germ line, I could engage with the experience of learning and research simultaneously by “accidentally” encountering unacknowledged boundaries separating each field and its respective version(s) of gametogenesis and germline stem cells.

### **Character Sketch: TAF4b**

TATA box binding protein associated factor 4b (TAF4b) is a gene that gives rise to a protein product integrated within a larger transcriptional regulatory complex. The entire TFIID complex, of which TAF4b is a small part, is understood to involve a multitude of protein-protein and DNA interactions (the nature of which are outside the scope of this paper). The way that Drs. Freiman and Johnson understand TAF4b’s function and homology is covered at length in the following chapters, though notably, this protein’s structure receives little attention throughout the course of laboratory experiments. Instead, protein function and form are inferred through phenotypic manifestations (visible organismal traits), and transgenic manipulations to include what are known as “reporter genes.” Thus, developing a cursory vision of how Dr. Freiman envisions the material structure form, and relational network of TAF4b to other genes and other transcription factors is crucial to conducting a valid ethnography of researchers who claim to study the “molecular etiology of fertility” (“Researchers at Brown: Richard N. Freiman Associate Professor of Medical Science”).

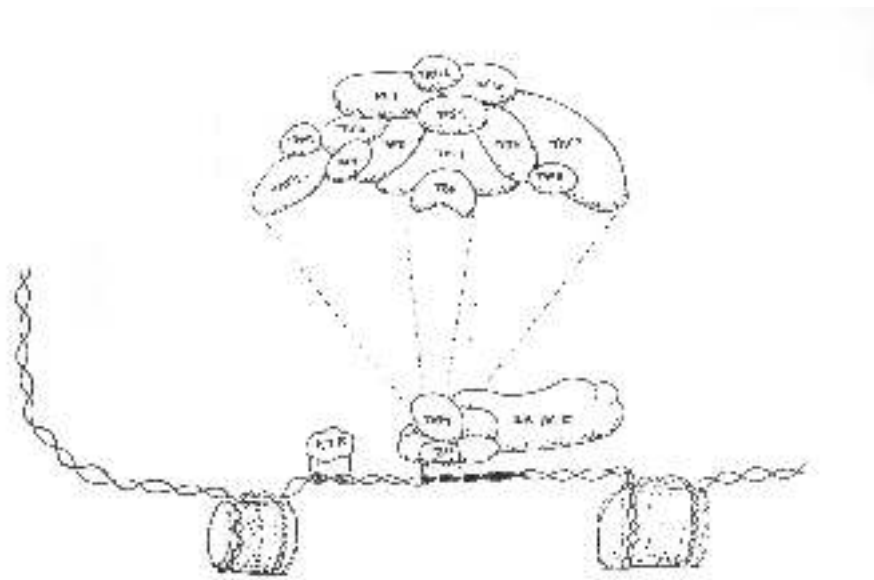
Much of the published literature characterizing the transcriptional function and complex interactions of TFIID transcriptional machinery originates in the lab of Dr.

Robert Tijan, a former mentor and Postdoctoral advisor of Dr. Freiman. In a description of the lab, molecular biology, the “Biochemistry of transcriptional chromatin transactions” appears first in the list of topics investigated- even before the study of cellular “stem cell pluripotency” (“HHMI Janelia Research Campus, Tijan Lab”). As described in previous publications, two identical TAF4b proteins are integrated into what is known as a “stable core sub-complex.” The association of TAF4b with additional proteins including the TATA box binding protein (TBP) is collectively referred to as the “Holo-TFIID” complex (Wright, Marr, and Tjian). In association with the RNA polymerase II machinery, this Holo-TFIID complex acts at the nexus between DNA and RNA and is assumed to undergo a conformational change (a change in shape) in the presence of TAF4b, enabling germline specific transcription (Freiman, “Spring TAF4b Structure Conversion”). Though TAF4b is not understood to be a definitive marker of germline stem cell identity in mice, it co-localizes with classic GSC markers including OCT4 at various points through out development of the mammalian gonad (Falender et al.; Lovasco et al.). Expression of the marker OCT4, a transcriptional regulator of stem cell totipotency and differentiation, is the most widely used biomarker in positively identifying mammalian germline stem cells (Pesce and Schöler). Note that the rough illustration I created for Figure 3, TAF4 subunits and TAF4b are understood to be interchangeable, and labeled simply as “TAF4.”

Increasingly, cryo-electron microscopy seeks to reveal the structure of the TFIID complex. Freiman instructed me to not dwell on the structural forms produced by cryo-electron microscopy because many structural predictions are approximate, contested, and “over-simplified” versions of reality (Freiman, “Spring TAF4b Structure Conversion”;



Papai et al.). In Figure 3 I sketch a rough, summary image that combines and adapts Wright et al. 2006's visualization (Wright, Marr, and Tjian) with a figure Freiman drew for his postdoctoral work in the same lab ("HHMI Janelia Research Campus, Tjian Lab"). In this representation, the TFIID complex is shown associated to RNA Polymerase II, positioned such that the hypothetical "TATA box," a DNA sequence that signals the "start" of gene's coding sequence (the dark section of DNA in this sketch) is aligned with TATA Box Binding Protein (TBP). A more detailed representation of the complex is sketched above, and notes the approximate theoretical positions of several other TAFs, or Transcription Associated Factors, within the Holo-TFIID complex. "Upstream" of the TFIID complex, that is, to the left, the presence of a transcriptional activator (ATR) is drawn, binding a hypothetical "promoter region" whose function is to enhance the transcription of the downstream DNA.



**Figure 3:** My reinterpreted sketch of TAF4b protein within the TFIID complex, based on a figure produced by Dr. by Freiman during his time in the Tjian Lab

Though this rough representation shows a singular imagined arrangement of material objects, TAF4b as a protein became increasingly abstracted in the PGL laboratory context. Day-to-day discussions placed the *Arabidopsis* TAF4b homolog in the realm of mRNA and DNA sequence, somewhat removed from its alternate identity as a protein “product. In the first series of computational experiments, TAF4b was understood in the context of amino acid sequence data, the stand in and arbiter of evolutionary conservation through time. In experiment two, TAF4b was alternately contextualized as phenotype, the total number of viable seeds per silique (*Arabidopsis* seed pod). Finally, experiment three reveals how, through transgenic manipulation, the TAF4b gene can transform into a highly engineered “reporter” gene with a green fluorescent tag. Consequently, the structural protein form of TAF4b depicted here in fact constitutes a single vision of an array of possible representations and contextualizations. These varied relational identities show how a singular gene in what is understood to be a complex network of processes takes on multitude of forms. This multiplicity and network complexity typifies how gametogenesis and GSC related genes exist as dynamic entities in cell and molecular biological frameworks.

### **The *Arabidopsis* TAF4b Question in Three Experiments**

This report, composed of a heterogeneous mix of molecular biological assays and critical theory, is loosely structured around three distinct experimental questions posed in the PGL between May 2014 and April 2015. The title page of each chapter features a hand drawn note created by Mark Johnson in a 2014 meeting. Each note was created with the intent to to illustrate basic questions and methods underlying experiments. These

figures give key insight into the “black-boxed information processing” of research scientists, the aspects of scientific life that gives rise to experimental design but may not be recounted in formal publications (Cetina). Sketches are included at the outset of each section to illuminate the narrative culture and the necessarily visual communication style of geneticists and molecular biologists.

Chapter two, “Deep Homology and the Reproductive Genome” introduces the central figure of experimentation and ethnography, the gene TAF4b. This “character” in the story is understood to function in mice as a regulator of transcription, that is the transcription of DNA sequences into messenger RNA (mRNA). This first experimental question employs computational biology in the assessment of TAF4b sequence similarity, and asks: How does the *Arabidopsis* TAF4b homolog compare to TAF4b sequences found in other organisms? An examination of genetic databases involved in producing this analysis suggests that gene origin and function are in fact contingent events. Additionally, this computational experiment allows for limited speculation about the evolutionary origins of such a protein, and hints at how sequence-level comparisons illuminate homologous gene function in diverse organisms and laboratory cultures.

Chapter three, “Enter the Gametes: Relating the Mammalian and Plant Germlines” introduces historical background about the development of germline stem cell theory before the turn of the 20<sup>th</sup> century. This historical account, situated before the genomic revolution, is then related to contemporary definitions of the mouse, rat, and *Arabidopsis* germ line stem cell population in an effort to reveal how structuring process persist and evolve through time with the establishment of each model. Evidence is synthesized from laboratory field notes, ethnography, and quantitative data to illuminate

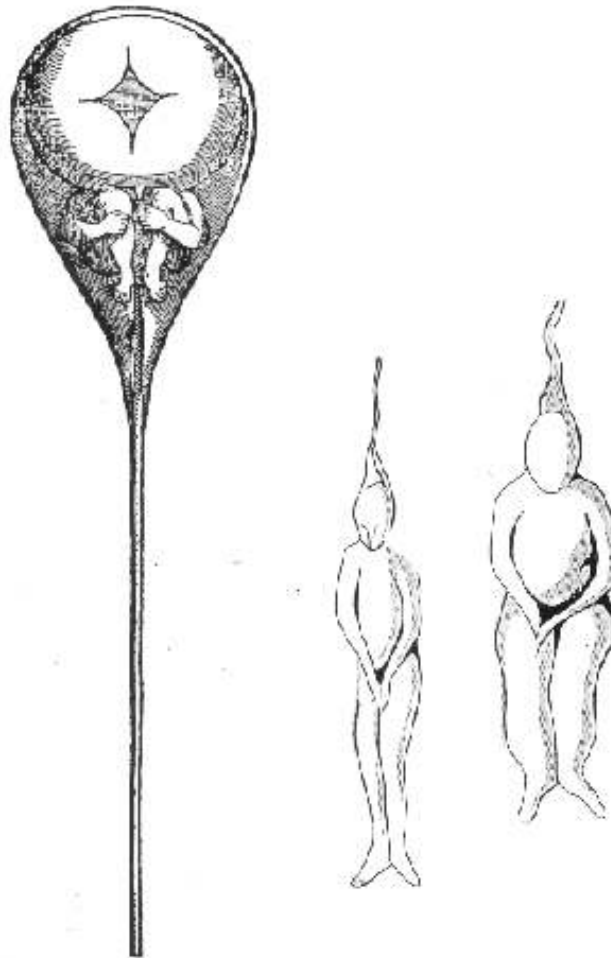
how TAF4b and the definition of “germline stem cell” are products of both historical paradigms and contemporary scientific conventions. The experimental question posed in this chapter is: Does a TAF4b deficient *Arabidopsis* plant manifest in any noticeable phenotype, and what might this phenotype (or lack thereof) indicate about the protein’s function in the plant germline?

Centered around biomedicine, stem cells, and questions of “vision,” chapter four asks how genetic engineering, including transgenic *Arabidopsis* produced in the course of PGL experiments, act to redefine historical notions of the germ line. The creation of transgenic “TAF4b:Green Fluorescent Protein” *Arabidopsis* allows experimentalists to ask: Where and when is TAF4b expressed in *Arabidopsis*? This experiment involved making a “GFP: TAF4b full genomic fusion,” e.g. an *Arabidopsis* plant that would have a green fluorescent protein easily visible under the proper microscope, attached directly to the TAF4b protein. The speculative and synthetic futures of the germ line as biotechnical and emergent life form are discussed. How does the growing power of synthetic biology render notions of the germ line as increasingly malleable, and what is the relationship of cell isolates *in vitro* when separate from their multicellular host?

I’m fascinated by the TAF4b “knock out” mouse’s phenotype, and the fact that “in plants and flies, there are also germ line preferential [specific] members of the TAF4 family” (Freiman and Johnson, “Winter TAF4b Meeting, PGL and Freiman Labs”). The fact that ancient, genetic homology may link in the reproductive machinery of plants, animals and unicellular eukaryotes in a material and social form raises questions about the origins of biological life. I hope to illuminate how this single gene, its function, and modes of characterizing germ line proliferative ability (stemness) vary lab to lab, model

organism to model organism. How can TAF4b, understood to have specific functional roles in mammalian sexual reproduction, serve as a reference point for comparison across tangential areas of scientific space and time? The *Arabidopsis* TAF4b homolog- whether an accident of convergent evolution, product of coincidental amino acid sequence similarity, or “variant” of ancestral “general transcription factors” - is the central node and organizing principal of this report (Freiman, “Specific Variants of General Transcription Factors Regulate Germ Cell Development in Diverse Organisms”).

## I. Historical Differentiation of the Germline



**Figure 4:** Nicolaas Hartsoeker's illustration of homunculi, tiny proportionate beings imagined to reside within sperm and eventually gave rise to human form (1695)

*“Although this disassociation of sex from procreation seems somewhat academic in organisms that reproduce only by sexual means, in plants and many lower animals sexual and asexual reproduction exist side by side, playing, presumably, very different roles. In a very different context this disassociation of sex from procreation came to have enormous social reproductions; it provided the biological backcloth for the ‘sexual revolution’ of the twentieth century” (Farley)*

A foreshadowing of sorts, the preliminary reading which Dr. Freiman assigned in his 2014 *Stem Cell Biology* course was August Weismann's "The Germ-plasm: a Theory of Heredity." Freiman's use of Weismann in his *Stem Cell Biology* course provides an invaluable starting point for analyzing how he, Dr. Johnson, and other 21<sup>st</sup> century molecular biologists understand the developmental biology and genetics of gametogenesis within the framework of historical accounts. This chapter investigates how historical biological conventions continually interact with the construction of germline stem cells of the 21<sup>st</sup> century. How and when did the germ line initially become centralized as an object of study accessible across model organisms? As suggested by Freiman himself, Weismann's work was formative in the development of stem cell and reproductive biology (Freiman, "Weismann Reading, Stem Cell Biology Undergraduate Course"). Freiman noted that as an undergraduate he wasn't encouraged to pursue the history of his field, but that it would be "really interesting" to engage with a historical text in the context of the class he was currently teaching. The Weismann reading fostered a lively class discussion about the methods, theory, and debate of scientific practice during Weismann's time. Further, students remarked on what they had perceived to be a "rift" between the "plant people" and the "animal people." Interestingly, Freiman suggested:

RF: what I thought was fascinating was the scientists in the 1800's were a lot more educated than scientists are today-

SW: in terms of, across the field?

RF: yeah.

MJ: yes, much broader.

(Freiman, “Weismann Reading, Stem Cell Biology Undergraduate Course”)

Here, Freiman notes that although there may have been a rift between “plant and animal people,” he perceived 19<sup>th</sup> century biologists like Weismann as having achieved greater fluency across model organisms relative to 21<sup>st</sup> century molecular biologists. While I recognize his explanation as valid, I argue that it is the cross-species fluency on a genetic, macromolecular scale that in fact characterizes a predominant structuring force in 21<sup>st</sup> century reproductive biology. “Plant” and “animal people” are increasingly consolidating their research around biochemical, sub-organismal processes, many of which are conserved across species.

Freiman’s choice to include Weismann’s “*Germplasm*” is unusual given that most Professors of Biology in the 21<sup>st</sup> century are averse to assigning readings of historical texts. Lynn Margulis, a biologist credited with the discovery of endosymbiotic theory, notes that while working at MIT, “any article...older than eight years, it’s transferred from science to humanities; ...science is considered ‘now’” (Margulis, “RU-TV Circle of Excellence: Lynn Margulis, Biologist, National Medal of Science Recipient Interview with Jay Tischfield”). She argues that turning to “historical texts” is in fact necessary “in order to ‘re-understand’ the problem” (Margulis, “RU-TV Circle of Excellence: Lynn Margulis, Biologist, National Medal of Science Recipient Interview with Jay Tischfield”). In a similar fashion, Freiman noted that Weismann’s “germtracks” constitute a formative development underlying 21<sup>st</sup> century notions of stem cell biology; the “original stem cell” of sorts (Freiman, “Weismann Reading, Stem Cell Biology Undergraduate Course”).



Contrary to the perceptions of many scientists, Latour suggests that biology is not pulled forward by some ultimate truth but instead “pushed from behind” by historical structures and epistemologies that limit its scope in imperceptible ways (Latour and Woolgar). Thus, it is crucial to develop a cursory history of reproductive biology as it existed before the 21<sup>st</sup> century. Characteristic properties of the germline between 1600 and 1900 include an insistence on anthropocentric accounts of sexual reproduction, a privileging of the material units of sexual reproduction above the somatic body, and an epistemology of continuity, bound categories, and independently acting units. What evidence do historical biological accounts offer in illuminating these defining methodologies? How are these historical modes of biological knowledge production evolving in the “century of the gene” and at the dawn of synthetic biology (Keller)?

Early biological realizations of stem cells and the germ line are tightly coupled to religious faith and anthropocentric reproductive frames. Ideologies of a perfect, predetermined biological future stemming from a single act of creation characterize the majority of reproductive biology until the mid 19<sup>th</sup> century (Farley). Classic examples of what is known as “preformationism,” a set of beliefs describing an early material and temporal developmental origin and of the germline, include Hartsoecker’s Homunculus in the 1690s and Weismann’s germtracks. Each account placed the germline in a privileged and fixed category (Lawrence; Weismann). A second theme that arises in concert with faith-infused origin stories of the germline is the naturalization of gender roles. Across the centuries, the germline and gametes have provided a prolific set of tools for establishing societal norms governing reproduction, gender expression, political ideals of the nuclear family and race relations (Fausto-Sterling). Such tools include

Weismann's "determinants," Darwin's "gemmules," and a host of other socially determined "factors," that act in concert to produce a socially durable notion of the germline (Farley; Heywood). Additionally, reproductive organs, even those of plants, are privileged above the somatic tissues and subject to the dominion of religious imagination. Carl Linnaeus, the father of taxonomy, based his system of plant classification entirely upon what he understood to be their reproductive organs. During the 1650's he organized all 24 taxa around a "sexual system" of morphology which related the "number, size and method of insertion of their stamens," (Paterlini). During the same period, colonialism and the rise of greenhouse hobbyists in Europe led to the establishment of *Passiflora* (passion flower) as a model organism. Its vivid sexual organs, five stamens encircling a central pistil, were likened to Christ's five stigmata (Endersby). This coupling of religious iconography with plant reproductive structures produced stable terminology: today the pinnacle of the angiosperm's female organ is known as the "stigma." Four hundred years of scientific practice later, durable language links the study of reproduction to moralized and religiously infused origins.

The growing influences between the 19<sup>th</sup> and 20<sup>th</sup> centuries of the German university system and cell theory and were necessary factors in producing a "biology of reproduction," and definitions of germline stem and "stem cell. The term "biology" was coined round 1800, and signaled the beginning of the end for "natural philosophers," scientists who did predominantly field work and taxonomy. Consolidated around a manipulative and quantitative approach to the science of life, the "biologists" were distinguished from natural philosophers by their "experiment," (Endersby) which was increasingly conducted inside a laboratory setting. The effect of the German University

system and cell theory on 20<sup>th</sup> century biological science was profound and resulted in a shift toward laboratory based, increasingly molecular research. Formerly standard approaches to university teaching popularized outside Germany, mainly lecture, were eclipsed by laboratory practice and hypothetico-deductive methods (Farley). This developing culture privileged laboratory research as an engine of knowledge production in germinating university systems, and began the tradition of standardizing model organisms (and the research communities built up around them). One lasting effect is a rift between natural philosophy (ecology, taxonomy), a field historically focused on whole organisms and populations, and molecular biology, a lab-based science that interrogates life on a cellular and molecular scale. This “hostility” between laboratory scientists and “naturalists” (Farley) allowed, until recent decades, the evolutionary relationship among groups of organisms to be eclipsed by their utility as laboratory bound model systems. University systems that encouraged “silo-ing” of molecular biology from studies of ecology and zoology created the perfect conditions for the emergence of a myopic molecular biology in the 20<sup>th</sup> century in which evolutionary relationships became irrelevant to laboratory questions of cellular form and function. In this epistemic culture, germline stem cells became discreet and static cells and tissues, located in specific model systems. These particular model systems happened to privilege metazoans, specifically *Drosophila* and *Mus*, which were understood to designate their germline early in development. Thus, accounts of the germline were based on an increasingly limited and standardized set of model organisms for which biologists perceived diminishing value in comparing materiality across species. Cooper notes:

“In the taxa to which genetics restricted its attentions (diptera, including *Drosophila*, and vertebrates including mice and humans), the determination of the germ line in its difference from the somatic cells, occurs very early in development so that Weismann’s separation of reproduction from developmental (re)generation appears to be closely approximated. This separation, however, is less clear in plants and animals capable of regeneration, as Weismann himself was aware. What this institutional and theoretical bias left aside were those less amenable forms of life- plants, colonial invertebrates, and fungi- that reproduce asexually, through fragmentation, fission, or budding.” (Cooper).

Thus, sexual reproduction in basal metazons and plants was placed in a category apart, institutionally separated from the conventional studies involving what had become “classical” model organisms. Academic “silo-ing” and “boundary work” (Gieryn) between those who study whole organisms or between those who study macromolecules in institutionally unrelatable model organisms is only now falling out of favor. As whole genome sequencing and increasingly sub-cellular macromolecular accounts blur former concepts of scale, species and time, 21<sup>st</sup> century molecular biologists begin to revalue the evolutionary development of sex.

With the advent of cell theory came the establishment of a longstanding hierarchy in which germline became superior to somatic cells, and the explicit gendering of gametes and gametogenesis reflected and reinforced contemporary “social order” (Farley). Eighteenth century biologists generally assumed sexual reproduction was a universal biological process, and that gametes necessarily arose from a defined population of precursor cells in every organism’s body (Farley). This framing

subsequently primed biologists to interpret parasexual and asexual reproductive mechanisms as “alternate” relative to sexual reproduction (Margulis, *Origins of Sex*; Fausto-Sterling). The regenerative properties and flexibility of germline as observed in organisms like hydra or bisexual (monecious) plants were most often seen as challenges to dominant theories and ignored or explained away (Cooper; Fausto-Sterling). In an interview, Dr. Wessel noted that Hans Driesch’s iconic experiments involving the separation of early sea urchin blastomeres were so unbelievable and disturbing that they drove Driesch from biology. When separated blastomeres subsequently regenerated multiple new, individual bodies, “Driesch left science to become a monk” (Wessel). Regeneration and the creation of new life without the familiar punctuation of sex were generally outside the purview of biologists who claimed to study reproduction.

For Darwin, comparisons between species of barnacles living either as hermaphrodites, sexually dimorphic male “parasitic” species, or progressively more free living male barnacles served as proof of the advances of sexual reproduction over asexual reproduction. He noted that there were some species in which the male “lived inside the females’ shells, almost like parasites...the males little more than tubes of sperm.” This observation led him to believe that asexual reproduction was an early and vestigial form of birthing subsequent generations, a belief shared by contemporary theorists including Lynn Margulis (Extavour and Akam; Margulis, *Origins of Sex*). Thus, the belief that sexual reproduction requires a higher, more evolved form than asexual production is durable, and reflects persistent attitudes about the high value ascribed to gametes relative to their somatic cellular counterparts.

The term “germline,” and theoretical “assumption of a contrast between the *somatic* and the *reproductive* cells,” was most famously defined by August Weismann in “The Germ-plasm: a Theory of Heredity” in 1893. He suggests that this defining separation between germline and soma is observable “in all multicellular plants and animals, from the most highly differentiated forms to the lowest... amongst the colonial Algae” (Weismann). Weismann’s germplasm theory, in which the material identity of germ line and soma cells are necessarily and reverently separate across the animal kingdom, is typical of one dominant paradigm that emerged and structured scientific dialog for centuries. His “germ-tracks” relied on “preformation” theory and assumed that a “preformed” and inherent germ line identity was maintained across generations of organisms through their cells by a material entity, the “id” (Weismann). This concept emerged in opposition to the “epigeneticists,” including William Harvey, who had speculated decades earlier that “God personally intervened in the development” of the embryo (Endersby). Seemingly positioned as a debate between secular “preformationists” versus religious “epigeneticists” in the 1800s, the beliefs of these two groups were initially equally religious yet differed in their biological explanations for genesis. For example, preformationism in Niccolus Hartsoeker’s time (late 1600s) meant the existence of “homunculi,” or microscopic but proportionate human beings within germ cells (Farley). As Endersby suggests, preformationists during Hartsoeker’s era believed “there had only ever been one act of creation: when God created Eve, she had all of humanity packed into her ovaries, one inside the another like an almost endless series of Russian dolls.” These speculative imaginings of the germ line are persistent and fundamental structuring forces of biology. In total, these divergent accounts of gametes and

gametogenesis act to privilege the germ line above the somatic body as a sanctified and elemental unit that is necessary for all life.

Paradoxically, the “rediscovery” of regenerative model organisms like hydra in the 21<sup>st</sup> century hints at the durability of historical debates around epigenetic (inductive, developmental) and preformationist (genetic determinist, inherited) theories of gametogenesis. In 2003, Extavour and Akam defined metazoan germline stem cell origins relative to the Weismann barrier. In their contemporary account, preformation is characterized by the localization of “materially inherited determinants before, or immediately following, fertilization,” while epigenetic designation of the germline stem cell population occurs as “a result of inductive signals from surrounding tissues” (Extavour and Akam). Marine invertebrates remain at the center of this debate, including planarian and hydra, and in fact catalyzed 19<sup>th</sup> century disputes around taxonomy, comparative anatomy, and evolutionary origins (Stott). Eloquently, Farley paraphrases these fundamental historical debates, “Is embryonic development epigenesis or evolution? Is it the new formation of complexity or is it the becoming visible of complexity previously invisible to us?” (Farley). Preformationism and epigenetic theories created considerable tension during the subsequent Darwinian revolution, and contributed to the decline in the perceived utility of regenerative model organisms like planarian and hydra in pursuing questions of the germline. Reacting against an epigenetic and Lamarckian tradition, Thomas Hunt Morgan and other 20<sup>th</sup> century biologists made certain that “phenomena of regeneration” would be safely sequestered in the domain of embryology (Cooper).

In studying the cells that constitute a germline, scientists have also historically made observations that act to naturalize and actively determine gender roles (Fausto-Sterling). Historical precedent dating back to Aristotle repeatedly linked “the higher faculties” of “character and behavior” with sperm, a “uniquely male contribution” (Endersby). Later assertions from both sides of the ovist/spermist debate, warring groups in the 17<sup>th</sup>-19<sup>th</sup> centuries that sought to determine whether egg or sperm alone ultimately contained the capacity for life, acted to naturalize the female body as sole locus for fecundation. Biologists who ascribed to “spermist” theories suggested that sperm possessed the basic capacity for new life, and upon fertilization transferred a complete embryo to the zygote. Ovists suggested the opposite: while a complete embryo was understood to be present in the vegetative, fecund egg, sperm provided the vital material force of the germline that endowed a female’s otherwise lifeless egg with life. Biologists ensured that women were “built up” around their uterus, especially in the 19<sup>th</sup> century (Farley). Such theories were also generally anthropocentric, with analogous accounts of sex involving gendered male and female roles appearing in the study of plant reproduction. Debates about the respective contributions of sperm and egg to the zygote have since evolved into 21<sup>st</sup> century discussions of protein-protein interactions, maternal mRNAs, and hormones, marking a departure from germline as gendered and distinct cell or tissue type. (For a comprehensive account of how gametes and sexual reproduction become gendered, see Fausto-Sterling’s *Sexing the Body*)

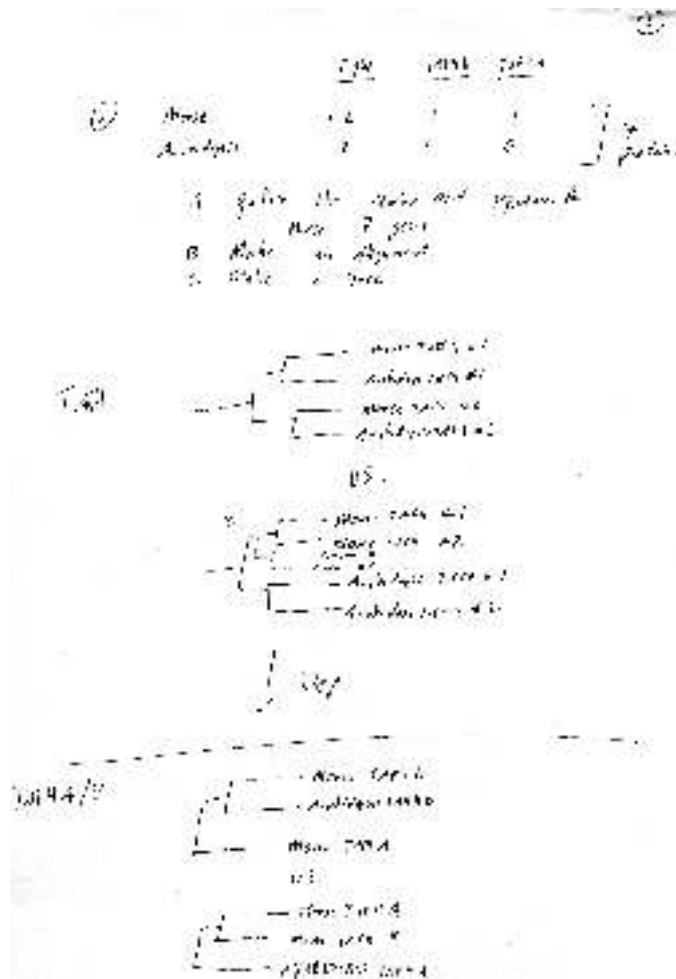
While early notions of the germline as a static and concretely defined cell population served to implicitly couple sexual reproduction to religious morality and social conventions of gender, 21<sup>st</sup> century definitions of the germline stem cells are



increasingly unstable and proliferative. Previously limited imaginings of anthropocentric, faith-infused, cellular entities relied on the estrangement of natural philosophy, taxonomy, and ecology from cell and molecular biology. Further, the insistence on dichotomies like a gametogenic origin that occurs exclusively through either preformation or epigenesis typify the relatively narrow conceptions of germline stem cells. Ultimately, the establishment of model organisms allowed for entrenched and myopic accounts of the germline to become conventional, structuring the epistemic culture of reproductive biology for centuries. Historical modes of biological knowledge production involving the germline are rapidly evolving in the “century of the gene,” and at the dawn of synthetic biology they are forced to incorporate an ever expanding number of incongruous definitions (Keller).

Accounting for the structuring force of history in giving rise to varied scientific accounts of germline stem cells is essential. The comparative accounts of Weisman and Wessel, for example, vividly demonstrate a transition from dichotomized biologies of germline/soma to a network-biology and macro-molecular understanding of the germline. In my developing research, I have found evidence that historical, gendered and anthropocentric accounts of gametogenesis are breaking down because their utility is increasingly eclipsed by non-traditional models and synthetic biological engineering. The study of parasexual reproductive strategies, bacterial conjugation as sex, and stem cell engineering are destabilizing historical notions of a materiality gendered sperm, egg, and germline. In addition to their shared past, mice and *Arabidopsis* are linked by a speculative future: much anticipated potential for patentable biotechnology and medicine.

## II. Deep Homology and the Reproductive Genome



**Figure 5:** Mark Johnson sketches out the goals and form of experiment one, a computational comparison (alignment) of different species' TAF4b and TAF4 amino acid sequences

*"Imagine stacking the representations on top of each other, enlargements on top of miniatures, larger and smaller than the average-sized original scale portrait; the pile can reach to the moon or even to infinity since there are no limits to size in either direction, except for practical ones...the card or photography right in the middle reveals the full-length portrait of the young lady, the zone above increasingly refined close-ups; the zone below, increasingly distant bird's eye views, leaving room for a growing crowd of beauties... Imagine pathways going from one portrait to another within the pile, a set of transversal paths in the cone or prism, linking together the various dimensions of a particular space...as a result, our vision is immediately transformed and turned upside down. The beauty lies next to her component parts: tissues, cells, large molecules, or otherwise in the middle of her tiny twin or cloned sisters. In the midst of her elemental composition and her possible reproductions." (Serres)*

This is an age where organisms are subsumed beneath their cells and macromolecules. Increasingly scientists concerned with germline stem cells make cross-species, *organismal*, comparisons, despite the fact that entire fields have emerged around “classical” model organisms, and many biologists would consider themselves “fly people” or “mouse people” (Freiman, “Spring TAF4b Structure Conversion”). Paradoxically, the myopic focus at a subcellular level tends to obscure the body of sexually reproductive organisms entirely, and somehow still invites cross-species comparisons. What is to be gained from an evaluation of the evolutionary tree, the branching and relation of extant species? Further, “sex” and “reproduction” were once imagined to be tightly coupled processes. Contemporary accounts tend to increasingly describe them as contingent, varying greatly among and between genotypes and species at the cellular and organismal level. Insight into the evolution of sexual reproduction destabilizes static definitions of germline and stem cell, forcing a reevaluation of sex, reproduction, and scale.

The underlying tension invoked by scale-sexual reproduction at the organismal and subcellular level- is made tractable by mapping a gene’s evolution through time. Underlying all TAF4b laboratory experiments is the basic question of evolutionary similarity, “conservation,” in genetics terminology, between transcriptional regulation of plant and animal sexual reproduction. We began the laboratory investigation with a computational genetic approach, the assembly of alignments and trees. In effect, experiment one involves “stacking the representations” of TAF4b on top of each other, allowing for a mingling of bodies across evolution. Through these analyses, we imagine the germline stem cells in relation to this “stack” of component parts (Serres). How does

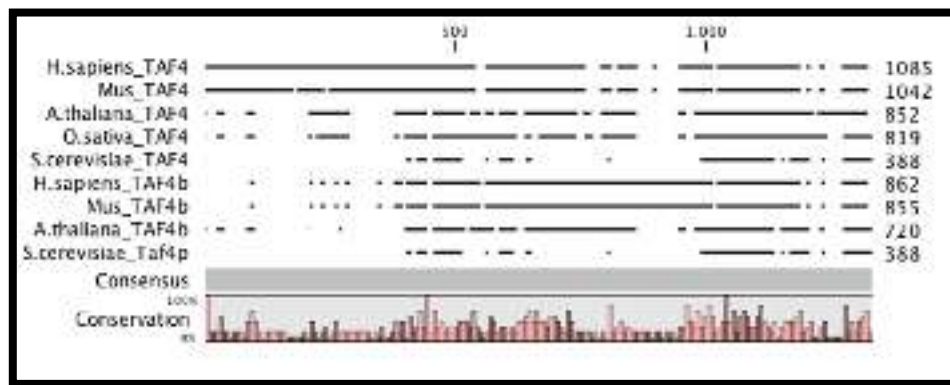
discord between molecular or organismal accounts of sexual reproduction emerge through quantitation of protein homology, and what conventions of pluripotency, regeneration, and sex are destabilized in the process?

My first instruction from Drs. Freiman and Johnson was to construct “alignments and trees” (Freiman and Johnson, “Initial Planning Meeting for TAF4b Pilot”) utilizing data available on NCBI’s database GenBank. Cetina noted in 2009 that the “genome project” and whole genome sequencing had not “brought molecular biology closer to the big science way of working” (Cetina). Subsequent proliferation of publicly curated databases in recent years, including NCBI’s GenBank (utilized heavily in this investigation) represent a massive, centralized effort supported by distributed, but highly collaborative, international actors. Genome science is big science.

“Trees” are an enduring and universal cognitive tool employed in the study of evolutionary, reproductive, and stem cell biology. Whether for mapping the evolutionary relationships of clades of organisms or relating the quantitative similarity of amino acids in homologous proteins, trees are both visually and symbolically ubiquitous. Possibly the first occasion for which trees served to explicate somatic and germ cell fate was Weisman’s “*Germplasm*.” During the undergraduate *Stem Cell Biology* seminar, Freiman remarked on Weismann’s tree figure: “So, this is a real tree he drew in 1892, and, it’s based on hypothesis, it’s not really based on- you know- rhetoric. It’s just what he’s *thinking* is real” (Freiman, “Weismann Reading, Stem Cell Biology Undergraduate Course”).

In creating the following figures, I am forced to assume that the quantitation of available data about the *Arabidopsis* TAF4b homolog- i.e. the amino acid sequences- is

both accurate and unbiased. Because I can not definitively assert that either of these two qualities are “real” from my own experimentation, like Weismann, the tree I produce is “just what I’m supposed to *think* is real,” a hypothesis following naturally from the logic of a computational algorithm bound by existing scientific knowledge. Keller notes that scientific data are assumed to “speak for themselves” though, in fact “all data presuppose interpretation” and “interpretation requires a sharing of a common language” (Keller and others). Post-translational modification, single nucleotide polymorphisms, and the “local genetic dialect” of a laboratory can influence what amino acid sequence is credited as



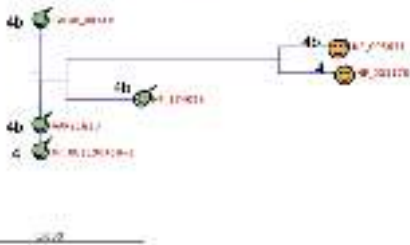
**Figure 6:** TAF4b alignment produced by CLC sequence viewer; graph at bottom shows percent conservation (similarity) across all sequences; corresponding species identity noted on leftmost side, y axis

being “accurate” and of “curatorial” quality. Newly created or “provisionary” genes and their amino acid sequences originate via laboratory experimentation, computational biology, and a socialized process of curation on various public databanks. This process of sequence curation is analogous to the creation of a dictionary or a thesaurus: the victors write the history. Though some open data movements rely on group consensus, curated genetic sequence data can only be produced by globally acknowledged labs with brand-name sequencers and recognized computational protocols, for which money and power are a precondition. The curation of expansive whole genome sequencing data is a highly

social enterprise involving human, animal, and cybernetic actors (Kirksey). The socialization of gene ontogeny is, as Cetina would suggest, “not merely there,” but in fact “capitalized upon and upgraded to become an instrument of scientific work” (29).

In genetics, the construction of an “alignment” involves comparing sequences of heritable material, either DNA, RNA, or amino acids, side by side, molecule by molecule. For the purposes of our first experiment, analyses at the sequence level attempt to reconstruct the evolutionary relationship among organisms, and give insight into the dynamic molecular mechanics of sexual reproduction across evolutionary time. Using a program called CLC sequence viewer we explored the transcriptional machinery of the plant and animal germ lines through alignments and trees that reflect the relative similarity (conservation) or evolutionary divergence of various eukaryotic TAF4b homologs (See Figure five, previous page).

AA Alignment and resulting tree  
TAF4 and TAF4b amino acid alignments for 4. Software and Hardware



**Figure 7:** Plant (green tear drop) and human (smiley face) TAF4b alignment data converted into a phylogram (tree) form

During the initial production of alignments and trees I was unaware of the distinction between “provisional/predicted” and “curated” amino acid sequences. When querying the GenBank database for TAF4b homologs, my searches returned a plethora of amino acid sequences purportedly belonging to yeast, oxen, monkeys, mice and men.

Failure to distinguish provisional/predicted from curated TAF4b amino acid sequences resulted in my first trees looking “wrong;” they didn’t reflect an intuitive prediction of evolutionary relationships (Figure 7). Simply learning to navigate the various databanks

and become fluent in their native dialect and labyrinthine particularities was initially quite inefficient and slow.

A caveat to computational-based analyses that predict evolutionary relationship among proteins/organisms resurfaced in December and challenged the underlying assumptions of our first experiments:

MJ: “I mean, the other thing, if you have time, writing up what you have so far- it would be useful in addressing the big hypothesis- is the trees. Like, ‘is TAF4b, TAF4b?’ Just because there’s a paper out there that says it is, doesn’t mean it is.”

(Freiman and Johnson, “Winter TAF4b Meeting, PGL and Freiman Labs”)

Here, Dr. Johnson acknowledged the alternately political and arbitrary nature by which a gene is born into 21<sup>st</sup> century databanks. Like any model that comes into being through analytical constraints and quantitative benchmarks, genes are a useful tool that often necessitates a flattening of detail to provide a useful operating paradigm. Further, the systems of measurement and benchmarks of positive identity defining a gene are embedded in preexisting frameworks of value, established modes of thinking. Based purely on quantitative sequence-level similarity, a gene and the protein it encodes might have predicted similarity in form or sequence, but in an organic body a gene’s function often eludes and muddles algorithmic prediction. Alternatively, a gene born from forward or reverse genetic screens in an experimental laboratory setting may encompass a multilayered history of scientific publication, historical presidents and personal advancement through namesake and publication.

MJ: ...back to your question about the structure. I'm not saying that these aren't all TAFs, and if you had the structures of all of them, I bet they would be structurally similar, um

SW: ...but, the way that they came into being...

MJ: The way that they evolved in each of these different lineages is, probably, different.

To add to the confoundedness, a final unsettling "problem" is that computational analyses remain inefficient in discerning convergent evolution events or "gene duplication."

SW: I...read a paper, and I think that TAF4 was duplicated initially, according to this paper, in plants- in early plants. Um. And now there's a TAF4 and a TAF4b in Arabidopsis, so...

RF: and that's the kind of evolution that very few people talk about, but I think they, it's harder for them to follow that kind of evolution. Right?

SW: Gene duplication?

RF: The molecular revolution focused on the amino acid begin everything, but...

MJ: He's talking about the possibility that somehow, under convergent evolution the expression pattern of these genes, um, there was some adaptive significance in expressing them specifically in the germ line."

RF: But if you can verify that they [TAF4bs] did evolve independently in these two different lineages I think that would be a major breakthrough.

(Freiman and Johnson, "Winter TAF4b Meeting, PGL and Freiman Labs")

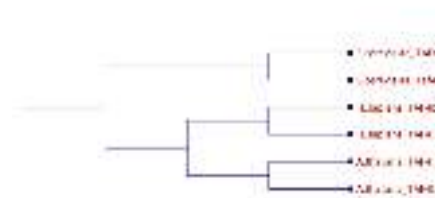


Several factors- the politics of career advancement through curating or naming genes, the arbitrariness through invisibility of automated computation - constitute the nebulous grounds by which homologous genes flare into and out of existence across species boundaries. Immediately after the discovery of DNA's double helix structure the "central dogma" structured molecular biology. In more recent decades, as Freiman noted, there has been a proliferation of "exceptions" to the "one gene one protein" dogma. These accounts exponentially complicate what was once a unitary, linear progression from DNA to RNA into translated protein (Geer and Messersmith; Keller). Understanding genes as a singular and static unit of measure vastly oversimplifies their place within an amalgamated landscape of test tubes, organic bodies, and social scientific lives of molecular biologists, evolving through time.

Later alignments and trees I produced utilizing strictly "curated" TAF4b and TAF4 amino acid sequences fit much better into our preexisting "cognitive maps" (Hutchins) of the gene's identity.

Of interest to Drs. Johnson and Freiman was how

TAF4b and the functionally related transcription factor TAF4 would "cluster" across a range of species. Using only "certified" scientific knowledge (Popper)- that is, the curated encyclopedic versions of TAF4b/TAF4 amino acid sequences available in GenBank- I produced the resulting tree (Figure 7). In it, all yeast, *Arabidopsis*, and human transcription factors cluster by species. What information does this figure communicate to geneticists Freiman and Johnson? Primarily, their interpretation of the tree above is as follows: species specific similarities in TAF4b and TAF4 transcriptional



**Figure 8:** TAF4b cladogram produced by CLC sequence viewer from curated TAF4b sequences

machinery exceed homologous conserved structural similarity across evolutionary time. Thus, we hypothesized that both TAF4b and TAF4 diverged as paralogs in their form and sequence in the distant evolutionary past, subsequent to the split of plants and animals from a common unicellular ancestor.

Recognizing protein and gene sequence homology between evolutionary distant organisms allows for exploration of what is known as deep homology. Deep homology, slowly gaining acceptance as a lens for molecular biological and biomedical study, allows researchers to acknowledge and cogently evaluate divergent reproductive strategies at the sub-cellular, macromolecular, genetic scale (Natterson-Horowitz and Bowers). This includes such diverse forms as parasexuality and microbial “conjugation,” modes of exchanging DNA and “germ line determinants” not formally acknowledged within dominant paradigms of gametes and germline stem cells.

Increasingly, focus in the field of stem cell biology has shifted to questions of “evolutionary origins” (Agata et al.). This shift is reflective of a phenomenon of broadening of definitions in what constitutes a germline stem cell, attributable to the rise of evolutionary developmental biology (“Evo-devo”) and increasing development of speculative human fertility therapies (Ewen-Campen, Schwager, and Extavour). In their 2006 paper, Agata et al. suggest a direct evolutionary link between stem cells and asexual reproduction that likely precedes tissue specialization (i.e. soma). Though this particular publication did not make waves, it is typical of a growing focus on basal metazoans like planarian, thrusting into the light non-traditional model organisms whose significance to the study of the germline was previously discounted by 21<sup>st</sup> century reproductive biologists charged with producing biomedical “translatibility” (Wessel). Translatibility

of what many consider “basic research” involving model organisms like planarian is a precondition for funding in a biomedically driven granting environment.

Agata et al. highlight the flexibility of the planarian germline, noting that “sexualization” from an asexual to a sexual state can be induced simply through altered feeding conditions. Additionally, the separation of planarian or hydra bodies into multiple sections by incisions or agitation will allow for complete regeneration of multiple complete bodies (Solana). Acknowledgment of and investigations into these capacities fundamentally challenge social notions of bodily continuity reproduction, and regenerative capacity. In fact, scholars like Leo Buss suggest that the “modern alliance” of evolutionary theory and genetics necessitated a “forgetting” of these highly regenerative, basal metazoans (Buss) during the 20<sup>th</sup> century. Such organisms did not mesh with the existing paradigms defining germline stem cell and sex, which were based upon model organisms like *Mus* and *Drosophila* (Agata et al.; Cooper). Notably, the authors reflect on how planarian and hydran “asticity” counters historical “assumptions,” and “zoological bias,”

“It is commonly assumed that germ cell segregation during the early stages of embryonic development is a general rule in the animal kingdom.

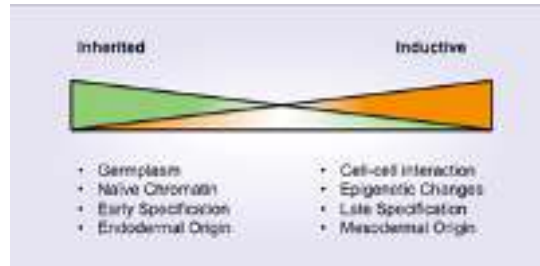
However, there are many animals in which germ cells are formed in adulthood, which is common in plants. In plants, stem cells in the meristem differentiate at the last stages of development, with the formation of flowers” (Agata et al.).

By this definition, the plant germline stem cell population is both exceedingly transient and exceedingly plastic in comparison to the durable, early-established stem cell population of mouse. In plants, differentiated tissues may give rise to unipotent gametes

at various points throughout development. In a sense, their statement directly addresses similar assumptions underlying my experimental question, “does TAF4b modulate the plant germline?” More deeply, what is the germline relative to plants, mammals and animals like planarian that fall along a “spectrum” of reproductive strategies? For these organisms, many of which are considered “true hermaphrodites” (possessing both male and female gametes) or can reproduce asexually through fission, reproduction is not necessarily sexual. Due to the influence of “evo-devo” biology, model organisms employed in the study of the germ line have undergone a renaissance period of sorts over the last two decades. The rediscovery of the “immortal hydra” (Cooper) and other animals that exhibit both extraordinary regenerative and sexually reproductive capacities, as well as characterization of “new” sexually reproductive model organisms (Ewen-Campen, Donoughe, et al.), the redefining germline stem cells of 21<sup>st</sup> century.

Reproductive developmental biologists including Gary Wessel and Cassandra Extavour are expanding the scope of 21<sup>st</sup> century models to include “non-model organisms” (Extavour and Akam), diverse echinoderms and insects that often fall outside the paradigms of developmental biology established by *Drosophila*, *Mus*, and *C. elegans*. Their work alternately refutes and reinforces historical debates around the validity of “preformationist” and inductive mechanisms of germ line specification, though is consistent in imagining an underlying homology, or “ancestral pluripotency module” (Ewen-Campen, Schwager, and Extavour). Contrary to the Weismannian tradition, Extavour suggests that “epigenetic germ cell specification may be ancestral to the metazoan” (Extavour and Akam). During an informal interview, Wessel countered the dichotomy of epigenesis/preformation theories (inductive/inherited in the language of his

particular lab). He suggested that gametogenesis is a process that occurs on a “a continuum” between these two modes, crossing his arms in conversation to pantomime a figure from a publication (Figure 9, right) (Wessel; Seervai and Wessel).



**Figure 9:** Seervai and Wessel’s depiction of germ line specification, a “continuum” between inherited and inductive germ line specification, reproduced from: Seervai, Riyad NH, and Gary M. Wessel. “Lessons for Inductive Germ line Determination.” *Molecular reproduction and development* 80.8 (2013): 590–609. Print.

While Wessel questions the dichotomy of inductive versus inherited mechanisms (Wessel) and Extavour

suggests that epigenesis has a distant evolutionary past (Extavour and Akam), Jordi Solana of the Max-Delbruck-Center for Molecular Medicine of Berlin Germany yearns for an entirely preformationist account of gametogenesis in which Weisman’s theory of germplasm continuity remains intact (Solana; Weismann). Solana’s germline is defined as “the line of cells that carry the genetic material from one generation to the next” (Solana). Using the highly regenerative model organisms hydra and planarian as examples, Solana argues that preformation is in fact a universally occurring process that employs a population of “primordial stem cells,” “PriSCs.” Further, Solana suggests that,

“The root of the multiple controversies regarding the germline specification and its phylogeny comes from the exclusion of the PriSCs from the germline definition... The inclusion of all PriSCs in a more generalized germline concept would, therefore, eliminate the root of these controversies” (Solana).

Solana’s publication arises in opposition to exclusively preformationist or epigenetic explanations of the germ line, and seeks to unify ultimate consensus around a

preformationist, “primordial stem cell.” In an era where germline stem cells are far less static than Solana’s definition suggests, why does he seek a preformationist “fix” to these historically opposed theories? The notion of an inherited and privileged germ line determinant holds perceptible psychic weight. This theory has persisted for hundreds of years and remains a structuring (though ebbing) paradigm in the way that scientific knowledge is produced relative to the germline. Despite this fact, Wessel reported having not read Solana’s publication; the once and familiar notions of permanence inherent to preformationism are in flux.

Regardless of whether GSCs are understood to arise by preformation or epigenesis, inheritance or induction, 21<sup>st</sup> century scholars agree that “it is now possible” and inherently “useful” to speak of the “molecular modes comprising gene regulatory networks.” (Ewen-Campen, Schwager, and Extavour). The focus on a “conserved germ line multipotency program” (Juliano, Swartz, and Wessel) naturalizes a shift from historically organismal accounts of gametogenesis to biochemical accounts that privilege proteins families and gene regulatory networks. This very culture gave rise to the comparative framing that catalyzed my *Arabidopsis* TAF4b experiments. Emerging paradigms and granting structures support this “germline as biochemistry” perspective, and act to structure research communities around the sub-cellular events of gametogenesis (Ewen-Campen, Schwager, and Extavour; Ewen-Campen, Donoughe, et al.; Juliano, Swartz, and Wessel; Lasko; Jr et al.; Ewen-Campen, Srouji, et al.; Lynch et al.; Schlecht and Primig). Multi-page tables that catalog the “genes required by germ cells for development” (Extavour and Akam) are common to such publications, and include genes like *nanos*, *oskar*, and *Oct3/4*, regulators of pluripotency. Seemingly

endless articles obscure organismal identity under the “multiplicity of functions” of “ubiquitous” macro-molecular players, for example “DEAD-box helicases” like *vasa* identified in the 1990s (Lasko).

During a PGL lab meeting on February 20<sup>th</sup>, we discussed a paper by Liu et al investigating a gene involved in gamete fusion, HAP2 (Liu et al.). HAP2 encodes a protein critical for gamete fusion in organisms as diverse as unicellular algae like *chlamydomonas* and multicellular eukaryotes including *Drosophila*. This gene is central to the investigations conducted by Jen, a graduate student in Dr. Johnson’s lab. HAP2 is a continuous thread leading from Dr. Johnson’s postdoctoral research (M. A. Johnson et al.) to his ongoing work as faculty at Brown. Though the paper of interest detailed experimental protocols involving *Chlamydomonas*, a family of unicellular flagellate algae, our implicit purpose was to conceptualize this gene under the umbrella of fertilization phenomena across evolutionary boundaries. Species identity was not entirely irrelevant in the discussion, but conserved protein “motifs,” cross-species similarities in fertilization mechanisms, and discussion of “membrane fusion phenomena” eclipsed species-level knowledge. Hypothetical experiments to compare “chlamy” and *Arabidopsis* membrane fusion mechanisms were floated: “we could attempt a rescue experiment,” “it would be possible to collaborate with fly people,” “...develop a cell-free fusion assay.” Inherent to the commentary was an affirmation that sub-cellular and macromolecular phenomena trumped organismal processes; species and sex at the organismal level became obsolete under a dense morass of gene regulatory networks and indeterminate evolutionary histories.

HAP2, like TAF4b and innumerable other genes involved in sexual reproduction, structure research communities irrespective of model organism and gendered sex role. Scientific communities and political economies are readily built around biological phenomena, i.e. fertilization or germline stem cell regulation, and constitute the governing syntax of molecular biology in the 21<sup>st</sup> century. A growing family of genes including *nanos*, *piwi* and *vasa* unite specialists across reproductive biology, cancer biology, and developmental biology- a point that will be elaborated in chapter four. Additionally, Jen reported never having taken a course in plant biology or plant genetics prior to the start of her PhD. Instead, the unifying theme in her trajectory as a scientist revolved around ovules, ovulation and fertilization, initially in cows, and was both necessary and sufficient to launch her subsequent research career utilizing *Arabidopsis* as a model system (Forcina). Furthermore, Drs. Freiman and Johnson speak the common language of genetics, providing them with a common currency and array of discursive practices that supersede model organism. A landscape formerly occupied by the study of “plant reproduction” is now dotted with groups organized around the investigation of “mechanisms of invasive cell growth,” and “determination of cellular polarity” (“Researchers at Brown: Mark A. Johnson”).

Protists, colonial organisms, and even “non-model” multicellular eukaryotes increasingly capture the attention of molecular biologists. These organisms gain recognition because they engage in a wide variety of asexual, sexual and “parasexual” reproductive strategies that exceed most 20<sup>th</sup> century paradigms of “sex” and “reproduction.” It is important to note here that many plants, including *Arabidopsis*, are self fertile. This means that they produce both egg and sperm and can give rise to fertile



offspring when their gametes fuse. Additionally, various plant species are capable of undergoing apomixis, an asexual reproductive strategy known as parthenogenesis in animals. Despite this immense diversity, sex and reproduction have historically been studied as necessarily linked processes. How has a “close coupling” of sex with reproduction been maintained by the biological sciences, and what processes have sustained or challenged this dominant form of vision?

Lynn Margulis, comparative protistologist and philosopher, centered her academic career as a biologist around the question of unicellular life and the evolution of organelles (Margulis, “RU-TV Circle of Excellence: Lynn Margulis, Biologist, National Medal of Science Recipient Interview with Jay Tischfield”). Known most widely for her work developing the theory of serial endosymbiosis, she has also contributed to the field of reproductive biology by encouraging a reevaluation of the seemingly natural categories of “sex” and “reproduction” (Margulis, *Origins of Sex*). In her work, “*Sex: Three Billion Years of Genetic Recombination*,” she insists that “in most microbes, organisms from which we have descended, sex is quite separate from reproduction” (Margulis, *Origins of Sex*). By Margulis’s definition, “Sex is the formation of a genetically new individual” while reproduction involves “copying” and results in “additional live beings” (Margulis, *Origins of Sex*). Margulis suggests that unicellular life exists in forms outside the paradigmatic definitions of sex, reproduction and germline stem cell function established by eukaryotic model organisms like *Mus*. How do Drs. Freiman and Johnson understand unicellular organisms’ sexual reproduction? In the case of the gamete “fusogen gene HAP2, observed in the alga *chlamydomonas* and in *Arabidopsis*, homolog? More specifically, how do they view this diversity of reproductive mechanisms

in relation to *Arabidopsis* and other multicellular eukaryotes? Discussing my analysis of TAF4b homologs, Dr. Freiman suggested:

RF: ... the question might be to look at unicellular organisms- see what's going on there.

SW: Yeah, that'd be good.

RF: I know they go through meiosis, but...

MJ: So, right, unicellular organisms have kind of a...a transient germ line.

JF: You sound so confident in what you're saying. [Laugh]

SW: The 'plus and minus varieties...'

MJ: So like a germ cell biologist – someone who studies the germ line-

RF: [A unicellular organism] is a 'germ cell- *it couldn't have* somatic cells.

(Freiman and Johnson, "Winter TAF4b Meeting, PGL and Freiman Labs")

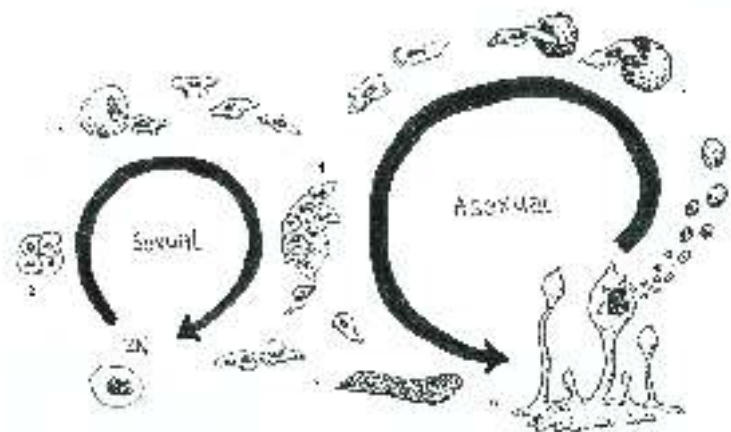
In his quip, "[it] is a germ cell," Freiman, like Margulis, suggests that a unicellular organism could be constitutive of its entire germline stem cell population, and thus capable of sex. This characterization calls for a subcellular definition of the germline constituted by macromolecules and homologous genes involved in syngamy and meiosis. Additionally, this exchange highlights the how the overly simplistic notion of naturalized male and female sexes actively excludes the reproductive processes of prokaryotic, unicellular organisms.

The dichotomy of gender as male/female is unsettled by definitions of unicellular organismal "sex." In Margulis' description of bacterial conjugation, "plus/minus" or "recipient/donor" relations characterize the exchange of DNA, not a static male or female identity. In her view of bacterial "sexual reproduction," conjugation occurs through the

donation of plasmids, circular extra-genomic DNA, across membrane extensions. Bacterial conjugation enables the creation of transgenic (genetically modified) plants as detailed in the fourth chapter, and constitutes potent means by which biotechnology is altering the germline of seed stocks across the globe. The study of unicellular and unicellular colonial organisms' reproductive strategies challenge the notion of "sexual" and "reproduction" as necessarily linked processes. Further, there is growing acceptance that lateral gene transfer (genetic exchange between bacteria and other organisms) is a major evolutionary force (Helmreich, *Alien Ocean*). Scholars like Margulis further insist on a rescaling of the germline to include the unicellular organismal scale, thus accounting for protists as evolutionary originators of "the meiosis-fertilization type of sexuality" (Margulis, *Origins of Sex*).

Margulis cites the unusual "parasexual" reproductive capacities of *Arcasia*, or slime molds, as additional evidence for need of clarification of the term "reproduction" and "sex." Blurring the evolutionary distinction between fungi and protist, *Arcasia* exist as alternately haploid amoebae and diploid zygote forms, and can engage in forms of asexual and sexual reproduction (respectively).

Their life cycle is a dynamic balance between haploid flagellated or amoeboid cells, which can undergo syngamy (nuclear fusion) to produce a genetically novel, diploid



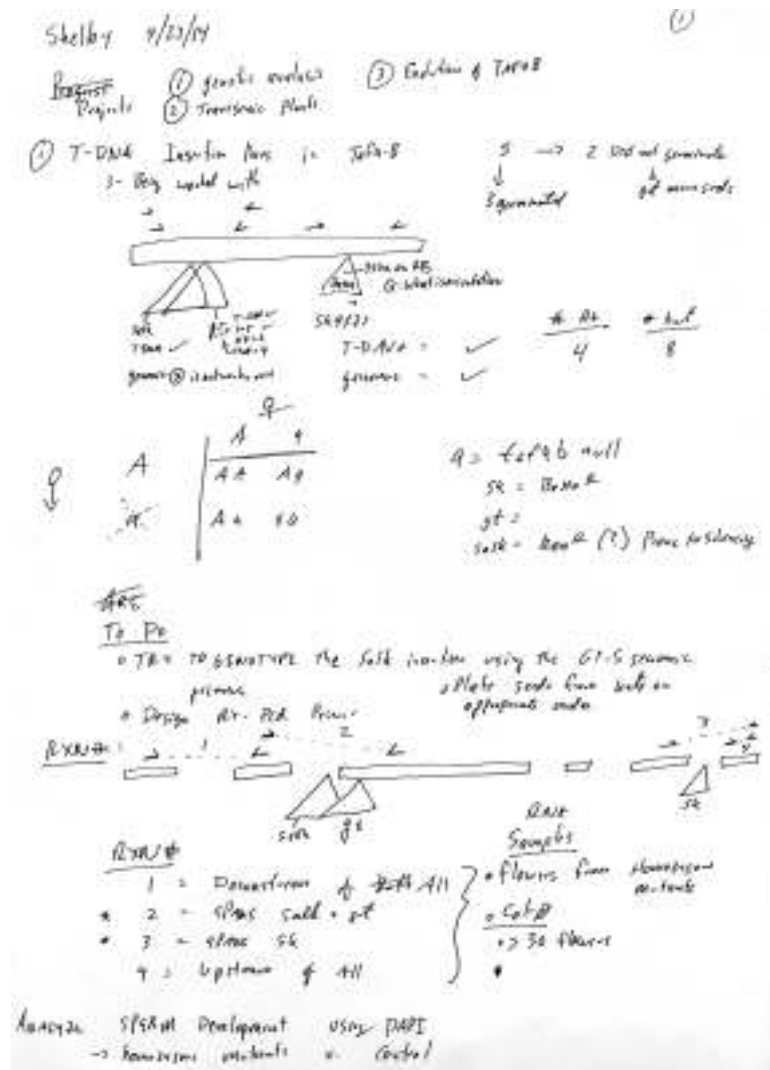
**Figure 10:** Parasexual lifecycles of the slime mold, note that it only exists in diploid form as a single celled "zygote" during stage one

zygote. This zygote subsequently gives rise to haploid, unicellular amoebas that can aggregate into a colonial, multicellular “slug,” and migrate collectively during times of resource depletion or high disturbance. Multicellular slugs, composed of unicellular colonial amoebas, can then transform into a “fruiting body” that allows mitotic divisions that asexually reproduce a generation of haploid spores. Notably, this alternation between asexual and sexual reproduction, marked by predominantly haploid forms is similar to the lifecycle of *Chlamydomonas*, the unicellular algae whose expression of the gene HAP2 caught the attention of Jen and Dr. Johnson. During the process of “sex” and “reproduction,” slime molds and *Chlamydomonas* engage in syngamy, blurring the line between “recombination” and “meiotic” sex. (Margulis, *Origins of Sex*; “Slime Mold Biology”; Gorelick and Carpinone).

An additional example of how deep homology pervades contemporary investigations into the germline includes Schlecht and Primig’s 2003 publication, “*Mining meiosis and gametogenesis with DNA microarrays.*” In this publication they identify “numerous” conserved meiotic genes held in common across a diversity of eukaryotes from unicellular yeast to mammals. In this publication, the concept of “gametogenesis” is considered in relation to unicellular organisms through the lens of meiosis, a form of vision I argue is unique to emergent 21<sup>st</sup> century definitions of gametogenesis and germline stem cells. This plurality of models, from *Mus* to *Chlamydomonas* and *Arabidopsis* gives rise to a dynamic and malleable definition of the germline that informs and often reflects “the technical ambitions of stem cell research” (Cooper). These questions inherently seek, as Solana’s publication title suggests, to close the “circle of germline and stem cell” (Solana).

Why are such broadened definitions of “sex” and “reproduction” historically underrepresented in both laboratory science and critical social theory of reproduction, despite their direct tie to questions of “stem cell” origin stories? Laboratory science defines gametogenesis and is generally anthropocentric and increasingly biomedical in scope. Reproductive biology has historically privileged anthropocentric and biomedical accounts of the germ line because durable historical precedent and speculative funding of biocapital have ensured that these definitions are the most useful and profitable tools in society. The applications of basic research involving protist sex or slime mold reproduction pales in its utility (and profitability) compared to the biomedical production of human zygotes through IVF technologies. The human germline stem cell population, especially in the case of women, is invested with social value by the nature of its perceived scarcity (E. Martin, “The Egg and the Sperm”). Further, understanding bacterial conjugation as free unrestricted sex- as opposed to deliberate means for transgenic manipulation- destabilizes its permanence as a cloning and GMO producing machine. Prokaryotic and unicellular accounts of reproduction are notable in their absence from majority scientific definitions of the “germline,” and speak volumes about existing norms that govern the field. Despite their historical invisibility, unicellular and “non-model organisms” (Extavour and Akam) have become crucial actors in constructing 21<sup>st</sup> century definitions of germline and stem cell. As their utility in biomedicine and speculative biocapital grows, unicellular organisms and parasexual beings will increasingly unsettle the myopic vision of reproductive biology and definition of germline stem cell.

### III. Enter the Gametes: Relating the Mammalian and Plant Germlines



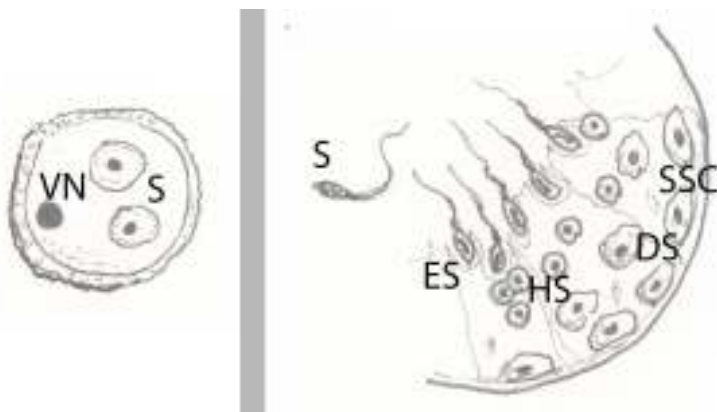
**Figure 11:** Dr. Johnson depicts experiment two, the result of a TDNA mutagenesis and reverse genetic screen. TDNA insertion sites are depicted as triangles dispersed across the TAF4b gene

*“So, this really brings the question of whether plants have a germ line at all. They really don’t in the way that mammalian people think of the germ line. It’s not something that’s set aside, you know, very early in embryo development. I mean, in flies, the germ line is set aside before the zygote divides.”*

(Freiman and Johnson, “Winter TAF4b Meeting, PGL and Freiman Labs”)

Determining the origin of the germline stem cells during organismal development, whether by gene expression assays, histology or genetic manipulation, remains a central preoccupation to those studying sexual reproduction. The molecular mechanisms and material logic of separating germline cells from somatic cells is a locus of social and scientific power, and gives rise to an increasing number of molecular markers and traits ascribed to germline stem cell populations. The ubiquity of mouse and *Drosophila* as dominant model organisms led to an entrenched belief that germline designation tends to occur relatively early in development across all taxa (Agata et al.; Extavour and Akam). In particular, *Drosophila*'s germline stem cell population has impacted narratives of gametogenesis for decades, privileging a preformationist account for germline origin (Agata et al.). Spermatogonial stem cells in *Drosophila* embryos are understood to arise immediately following fertilization via a population of cells called the “pole cells,” thus maintaining the “Weismanian barrier” and enforcing the belief that inherited or “preformationist” means determine the germ line- somatic divide (Illmensee and Mahowald).

Once established during development, the tissue architecture of plant and animal germline is strikingly different. While mammalian



**Figure 12:** Site of gametogenesis in adult male *Arabidopsis* and mouse, at left, Pollen cross-section: VN, vegetative nucleus, S, sperm nuclei; at right: seminiferous tubule cross-section from mouse: S, spermatozoa, ES, elongating spermatids, HS, haploid round spermatids, DS diploid spermatogonia, SSC, putative spermatogonial stem cells

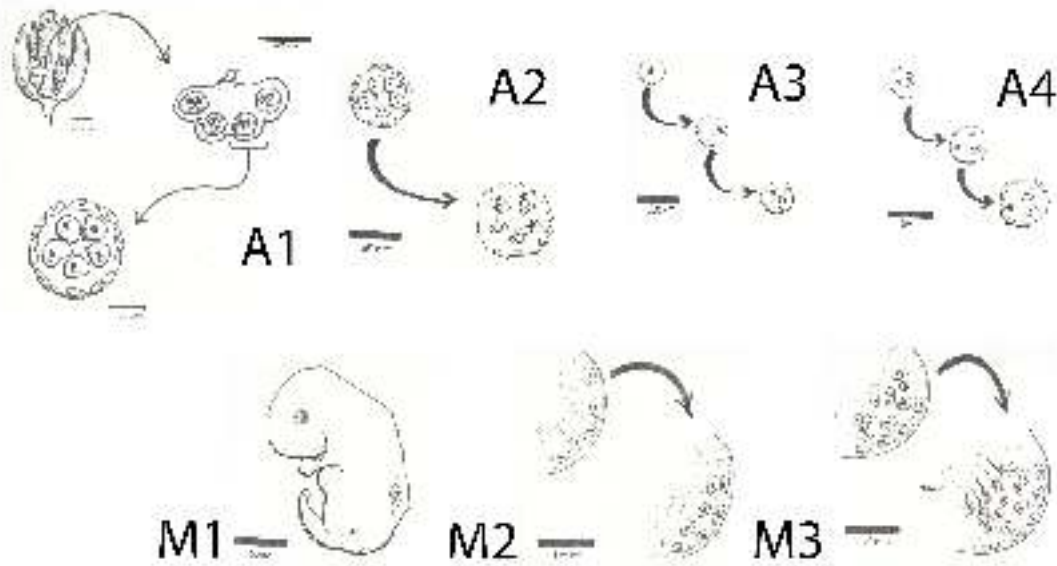
(Adapted from Hunter et al. and Sundberg and Østergaard)

sperm arise within a series of winding tubes called the seminiferous tubules of the testis (Figure 11, right), *Arabidopsis* spermatogenesis occurs within bicellular pollen, eventually producing sperm (tri-cellular pollen Figure 11, left) (Sundberg and Østergaard). Mature pollen contains two haploid sperm, one for fertilization of the egg and another for production of a triploid endosperm, and a vegetative nucleus (Yang and Sundaresan; Cardarelli and Cecchetti). The vegetative nucleus of pollen is responsible for directing the development of the pollen tube, a cell that functions in delivery of sperm for syngamy with the egg cell (Taylor and Hepler). Thus, while mammalian biologists understand fertilization to involve two cells, namely sperm and egg, plant geneticists understand the process as multicellular: egg and sperm plus the formation of endosperm (see appendix for more on this topic). Correspondingly, the descriptions of developmental events that give rise to tricellular pollen and spermatozoa in the seminiferous tubules vary widely.

Primordial germline stem cells that eventually give rise to the mouse gonad are said to arise as early as embryonic day 5, after which point they eventually migrate from the yolk sack to the genital ridge, colonizing the embryonic gonad (M1, below) (Pelton et al.). The terms “primordial” and “putative primordial germline stem cells,” reveal how developmental biologists seek to identify progressively earlier markers of germline “stemness” in metazoan models. Expression of the marker OCT4, a transcriptional regulator of stem cell totipotency and differentiation (Pesce and Schöler), is the most widely used criterion for positively identifying mammalian germ line stem cells and primordial germ line cells. TAF4b has not been widely used as a marker of germ line stem cell identity, but seems to co-localize with classic GSC markers including OCT4 at various



points through out development of the mammalian gonad (Lovasco et al.). Between embryonic day 13 and birth, mouse spermatocyte stem cells give rise to diploid spermatogonia near the basement membrane of the seminiferous tubules (M2, below) (Bellve et al.). In early life, pups become fertile with the onset of sperm production following formation of elongated sperm and mature spermatozoa (M3) (Yoshinaga et al.).



**Figure 13:** Gametogenesis in *Arabidopsis* microsporocytes beginning with floral stage 7 and anther stage 4 (A1), followed by meiosis II (A2), and development of polarized microspore into bicellular pollen through mitosis of the generative cell (A3), finally producing tricellular mature pollen (A4); Spermatogenesis and GSC designation in mouse seminiferous tubules, embryonic day (ED) 12.5-birth, (M1) depicts colonization of gonocytes around ED 12.5, (M2) mitotic proliferation of gonocytes to form diploid spermatocytes, followed by spermatocytes entering into meiosis at birth, (M3) haploid round spermatids develop into elongating spermatids and later mature spermatozoa; (Adapted from Hunter et al. and Sundberg and Østergaard)

In contrast to mammalian gametogenesis, most accounts of the plant germ line begin long after the embryo has matured and germinated, forming within an adult plant's differentiated floral organs. Evidence by both literature review and comments made by Dr. Johnson, most plant biologists agree that even the cells comprising the inflorescence meristem are not “germline stem cells” in the way that “animal people” use the term (Freiman, “Spring TAF4b Structure Conversion”). Following Meiosis II, the microspore

mother cells give rise to tetrads within the tapetal walls- support cells of the floral anther sack (male floral organ, see A1, A2 above). Next, the free microspores become polarized, and undergo pollen mitosis (A3). Finally, the generative cell of bicellular pollen undergoes pollen mitosis II, giving rise to mature tricellular pollen (Hennig et al.). Thus, the male gametophyte, or gamete bearing tissue, of *Arabidopsis*, is a tricellular entity composed of two sperm and a vegetative cell, whereas mouse spermatozoa exist as individual units. The underlying genetic homology drastically yet different reproductive anatomy of *Arabidopsis* relative to mouse enables both concrete insight through molecular genetic comparisons and additional instabilities in functional definitions of the gene TAF4b in sexual reproduction.

Accounts of the *Arabidopsis* “germline” in the 21<sup>st</sup> century assert that there are two reproductive lineages: the sporophyte (spore producing generation, pollen) and the gametophyte (gamete producing generation, the vegetative and flowering plant) (Evert and Eichhorn). This ontogeny traces back to the mid 1800s, and “generationswechsel,” or “alteration of generations,” a non-controversial concept in its modern form first described by Japetus Steenstrup and others (Farley). The gametophyte can be further characterized as possessing two committed lineages of stem cell, shoot apical meristem and root apical meristem, which later give rise to a multitude of specific cell types including inflorescence meristem (Schmidt, Schmid, and Grossniklaus; Evert and Eichhorn). In a recent review, Schmidt et al note that,

“Thus, unlike in most animals, where the germ line is set aside early in embryogenesis, the plant germ line is determined only late in development, during floral organ formation. Here, we consider the spore mother cells to be the first

cells of the germline, as the lineage of the gametes can unambiguously be traced back to them (Grossniklaus, 2011). However, it should be noted that, because gametophytes consist of both gametic and non-gametic accessory cells and the germ line is defined as the cell lineage that differentiates into gametes, some authors place the determination of the germ line later during gametophyte development to the immediate precursors of the gametes.”

(Schmidt, Schmid, and Grossniklaus)

Schmidt’s description of the *Arabidopsis* germline emphasizes the sperm’s relatively “late” emergence during floral organ development, and does not refer to the precursors as “germline stem cells.” The “spore mother cells,” diploid precursors to egg and sperm, are understood to be functionally analogous but late to develop germline precursor cells. Schmidt et al. note that the mechanisms of megaspore mother cell commitment to germline fate are as yet poorly understood.

A cursory development of both *Arabidopsis* and mouse models relative to the predominant paradigms of germline stem cell regulation reveals underlying tensions. What material characteristics of the model organisms *Arabidopsis* and *Mus* do Drs. Johnson and Freiman consider the most useful in their investigations? Examining both the history and the perceived utility of each model gives insight into how organisms become invested with value and relational meaning in 21<sup>st</sup> century study of gametogenesis and reproduction.

RF: “Mechanistically, the mammalian germline is a challenge to figure out...[my graduate student needs another reporter, GFP constructs of this or that]...but that’s another \$50,000 a year.”

MJ: “In Arabidopsis it’s really cheap: just sun and dirt.”

RF: “How much do they [the TAF4b mutant, null seeds] cost?”

MJ: “\$4.00!”

[Both laugh]

MJ: “No problem Rich, you can just give me \$2.00”

RF” “Yeah, I was going to offer to help fund but...the coffee was more expensive than the experiments!”

MJ: “If you opened up the growth room and said, ‘You’re free!’ nothing would happen, they’re [the plants] happy there...But mice have rights, they have to be treated ‘nicely.’”

RF: “I need to get a *real* model organism...”

(Freiman and Johnson, “Initial Planning Meeting for TAF4b Pilot”)

What is a “real model organism?” Does “real” exclusively imply “inexpensive” in this context, or properties like small genome size and quick generation time? Cetina suggests that the goals of “production systems” and “model systems” are inherently in conflict, but often “pursued at the same time” (Cetina). A production system requires specialization, an iterative process of trial and error and procedure-laden familiarity to perfect. Production systems also carry the distinction of giving rise to a predictable and consistent product, while model systems are imagined to bring equal parts novelty and predictability.

With these observations, a host of illuminating conversations about the nature of sexual reproduction in plants and animals followed. At what point during development do these organisms have a defined germline? How might a protein like TAF4b, shown to be

crucial in a mammalian context (Falender et al.; Lovasco et al.), be understood in the context of plant gametogenesis? The history of a dialectic between plant and animal reproduction predates even cell theory and microscope technologies powerful enough to visualize gametes (Farley). The products and processes of these investigations frame debates about gender and sexuality that extend far beyond the realm of laboratory bench work, model plants and mammals.

The study of gametogenesis in *Arabidopsis* and mouse is a product of historical processes that resulted in their establishment as both model organisms and knowledge production systems. How did each model become established as such, and what scientific worldviews- norms naturalized through years of scientific president and personal experience that may “preclude recognition of the force of language”- were assumed by Drs. Johnson and Freiman (Keller and others)? Johnson’s model organism of choice, *Arabidopsis*, came into the fore during the mid nineties concurrent with the rise of big science, whole genome sequencing and genetically modified organisms. Freiman employs a mammalian centric approach in his lab, utilizing various mouse and rat mutants with explicit emphasis on relevance to human health and disease. The culture that produced and standardized each of these models grew from a shared past, including the influence of the German university system, development of cell theory, and privileging of the units of heredity above the soma. This shared origin story has fundamental implications for the dynamic and speculative future of germline and stem cell biology, and delineates scientific descriptions of reproductive capacity, sex and gender across varied forms of life.

Following the evolution of germline stem cells from what was understood to be inherently male or female, unipotent entities into multidimensional, diverse and dynamic forms reveals fundamental instabilities in contemporary definitions of germline and stem cell. Factors that enabled this departure from previously dichotomized and essentialist notions of the germline include the consolidation of biotechnology as a profitable globalized industry, establishment of genetics as “big science” through whole-genome sequencing, and the rise of “flexible specialization” as a biologically rationalized and desirable socio-political order (E. Martin, *Flexible Bodies*). These factors subsequently allowed for an expansion in the depth and breadth of available model organisms, and increasingly encouraged scientific investigation organized around families of conserved, sub cellular phenomena and gene regulatory networks, i.e. membrane fusion (see chapter two, RE: HAP2) (Extavour and Akam; Forcina).

Coupled to issues of national politics and economics, the explanatory power of biology constitutes a formative social technology emerging in the 19<sup>th</sup> and 20<sup>th</sup> centuries. Many authors have suggested that Darwin “borrowed his metaphor of natural selection from industrial capitalism” (Endersby). Such notions allowed for both the naturalization of capitalism and the coupling of science and political economies in a novel way, with fundamental implications for the study of reproductive biology (Helmreich, “Species of Biocapital”). The development of *Drosophila* and Wistar rats during the 20<sup>th</sup> century demonstrated “what was possible when an organism was mass-produced” (Endersby), and allowed for unprecedented continuity across differential geographic and temporal engagements with science. This explosion of scientific productivity in 20<sup>th</sup> century academia has its origins in the ubiquity of university research systems modeled after

German institutions, and depends on “fusion of two ancient categories, life and machines” (Cetina). Standardization of both rat and mouse strains enabled a surge in translatable biomedical research of human diseases. Additionally, growing public-private partnerships, industry funded research, and emergence in globalized economies helped to distinguish 20<sup>th</sup> century biocapital from previous scientific endeavors (Helmreich, “Species of Biocapital”; Keller; E. Martin, *Flexible Bodies*). Endersby suggests that corporations including General Electric and DuPont recognized scientific endeavor modeled after the German university system as a means to restore economic prosperity stunted through lack of extensive empire. In addition, the “new molecular biology” of phage and bacterial genetics emerged, enabling unprecedented control of genome engineering and privileging the utility of small model organisms with short generation times and small genomes (Endersby; Keller).

Scientific investment in the standardization and socialization of model organisms through political and industrial networks continued with *Arabidopsis* beginning in the post WWII era (Keller). Initially discounted by Freidrich Laibach due to its tiny chromosomes- unsuitable for his studies of nuclear morphology and cytogenetics- *Arabidopsis* was “rediscovered” by the US Joint Intelligence Objectives Agency through an odd twist of fate. Subsequently, it was popularized by Chris and Shauna Somerville, Meyerowitz, and others, notably with the explicit goal of creating a research “community” around the plant (Endersby; Rhee et al.). The catalysis of the *Arabidopsis* Genome Project in 1989 enabled unprecedented public-private partnerships, including significant funding and data sharing by Monsanto and other biotech giants. Collaborative, networked efforts enabled creation of state-funded stock centers (seed banks) and set a

new precedent for the open data movement: experimentalists “loaded all the [sequencing] data on to a public database called GenBank, accessible via the internet, before they had even looked at it themselves” (Endersby).

Massive, publically accessible data made available through sites like TAIR (The Arabidopsis Information Resource) and GenBank enabled both proliferation and consolidation of the genetic explanations of *Arabidopsis* and *Mus* germline. This knowledge was enabled by massive forward genetic screens in several multicellular eukaryotes, during which mutagenesis is performed and resultant phenotypes of interest are subsequently analyzed in an attempt to find the genetic basis for the observed trait. These bio-social technologies gave rise to plants like my TAF4b-2 mutant from the Syngenta Arabidopsis Insertion Line (SAIL). Related processes also enabled Dr. Johnson’s postdoctoral work in characterizing HAP2 mutants.

The characterization of highly conserved metazoan embryonic GSC patterning genes including *vasa*, *nanos*, *Oct4*, and others also began in the late 70s through early 90s (Nusslein-Volhard, Frohnhof, and Lehmann) and employed mainly *Drosophila*, *C. elegans* and *Mus*. These new paradigms of gene families and pluripotency modules were crucial in establishing the 20<sup>th</sup> century model of gametogenesis and lent credence to the use of cell biomarkers in characterizing germline stem cells. Correspondingly, the beginnings of cross-species comparisons, “evo-devo” experiments, and recognition of deep homology in the genetics of gametogenesis was normalized.. Freidman’s 1999 publication “*Expression of the cell cycle in sperm of Arabidopsis: implications for understanding patterns of gametogenesis and fertilization in plants and other eukaryotes*” is typical of this trend. Characterization of the highly conserved "homeobox"



genes (Nirenberg and others) further encouraged study of the germ line through comparative embryology.

During this era, fundamentals of the plant germline transcriptional machinery were also consolidated. The fundamental theory of ABC models of floral organ development emerged at the turn of the century (Bowman et al.) (note that Meyerowitz is co-author). This theory postulated that a limited number of transcriptional regulators (subsequently determined to be what are called “MADS box” genes) led to development of floral organ identity; the designation of sepals, petals, stamens, and carpel. Characterization of these transcription factors, including “*APETALAI*” (*API*), led investigators to conclude that mutations of “AP” genes “disturb two phases of flower development, flower meristem specification and floral organ specification” (Bowman et al.). By this definition, disturbances in the processes of “floral organ” and “floral meristem specification” seem to suggest that transcriptional regulation defines the origin and limits of the plant germline in distinct phases and tissues.

Various *Arabidopsis* mutants exhibiting reduced microsporogenesis (pollen production) including *EMSI*, *SERK1/2*, and *TPDI* have been characterized since 2000 (Canales et al.; Yang et al.). These mutants shed some light on how microspore development may be regulated in the context of anther tissues known as “tapetum,” the support cells of plant’s male germline. Additionally, recent insights from both plant genetics and metazoan developmental biology increased interest in small RNA-based and epigenetic germline-specific regulatory pathways (Lasko). Both of these mechanisms and the epistemic culture of dynamic and systems-based biology they necessitate arose in contrast to earlier, 20<sup>th</sup> century one-gene-one-protein paradigms (Keller). For example,

the participation of *ARGONAUTE* (*Ago*) proteins in smallRNA mediated germline specification relies on a flexible and dynamic characterization of gametogenesis unimaginable to developmental biologists in previous decades (E. Martin, *Flexible Bodies*).

### **Characterizing Mutants**

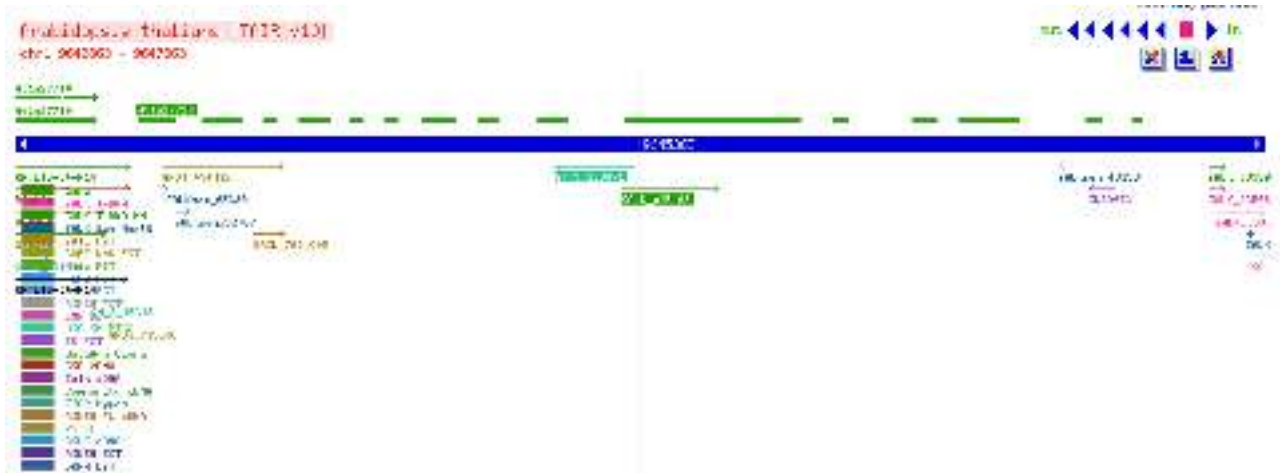
Lab research and ethnography continued as I became familiar with several essential techniques and protocols in the PGL. These included PCR, DNA extraction, and *Arabidopsis* care. These three aspects of work flow in the PGL form the underlying syntax that supports all other methods and enable the geneticists to ask questions. I worked primarily under Johnson's third year graduate student, Jen, and owe her a great debt of gratitude for supporting the project and engaging with my questions, even when they weren't directly relevant to lab activities. As I entered into the world of PGL, familiarized with the currency of lab exchange and improving in my fluency of genetics-speak, this first question involved what is known as reverse genetics. Reverse genetics is a process by which specific a gene locus is mutated and the resulting organisms are analyzed for a phenotype (a visible physical, functional or developmental defect).

The fundamental experimental questions posed in experiment two include: Does a TAF4b deficient *Arabidopsis* plant manifest in any noticeable phenotype, and what might this phenotype (or lack there of) indicate about the gene's function relative to the plant and animal germline? This second series of experiments involves the use of what I termed "TAF4b-2 mutant" plants from the Syngenta *Arabidopsis* Insertion Line (SAIL) stock center. This name was a simplification of the cumbersome stock center name, SAIL\_763\_C05, or stock ID# CS834119. We intended to use blind measurements of

phenotypic endpoints like seed count and RNA extraction to evaluate whether TAF4b-2 plants were in fact mutants. These plants were produced through a process called T-DNA mutagenesis, which involves random insertion of a piece of “transfer DNA” by agrobacterium into the *Arabidopsis* genome (Alonso et al.). The plants are “mutagenized,” when this TDNA interrupts a particular gene of interest and consequently decreases or inhibits its ability to be transcribed into functional protein. The emergence of massive stock centers which provide products like SAIL “mutant” plants constitutes a critical moment in the establishment of *Arabidopsis* as the dominant plant model organism.

The “reverse genetics” experiment began in early June when a shipment of *Arabidopsis* seeds arrived. These five “seeds lines” traveled from no less than four distributors. It is worth mentioning two of these distributors: their current form and function grew from a storied past and is reflective of genetics’ big science present and speculative future. The *Arabidopsis* germplasm distributors SALK (from the Salk Institute and Genomics Laboratory in LAJolla CA) and SAIL (Syngenta Arabidopsis Insertion Line) provided three of the five seed lines. The products these distributors provide and manage include a vast array of mutated seeds, allowing researchers to select specific genetic mutations to serve as a specific tool in their laboratory experimentation. This particular arrangement of plants and social structures is unique to the 21<sup>st</sup> century, the product of (somewhat paradoxically) emerging public-private collaboration and open data movements. Producing and cataloguing thousands of different mutations in such a manner is prohibitively time intensive and complex in a small academic lab setting, thus

providing this service for a fee filled a necessary and profitable niche in the biotech industry.



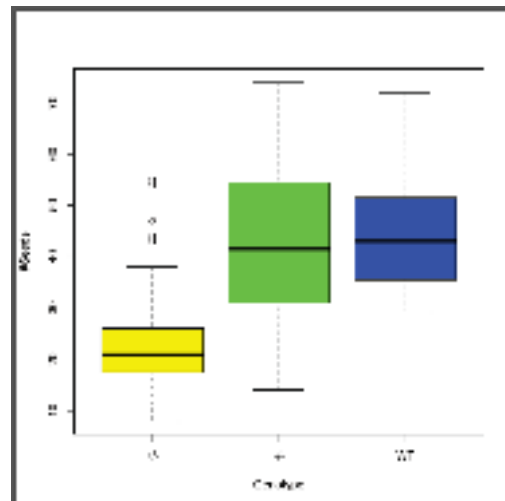
**Figure 14:** Screen shot from “TDNA Express” (<http://signal.salk.edu/cgi-bin/tdnaexpress>) depicting TAF4b gene (green lines at top) and a selection of available seed stocks with TDNA inserts interrupting the TAF4b gene at various locations. Note orange text “SAIL\_763\_C05,” top left, the TAF4b-2 mutant used extensively in this experiment

The reason each of these five seed lines were selected in the context of the PGL and TAF4b experiments was that each harbored a potential mutation in the TAF4b gene (See Figure 13). In the case of the SAIL line, seeds carrying what is known as a TDNA insertion, described previously. This insertion was created through the process of a vast and nonspecific “mutagenesis” through bacterial transfection with exogenous DNA. Transfection via *Agrobacterium* is a tool essential to plant genetics labs everywhere, and allows for the transfer and integration of a bacterial gene into the genome of the model plant. Generally speaking, these methodologies also form the basis of genetic engineering approaches used in producing GMOs. In the case of SAIL seed line, the bacterial transfection resulted in one piece of “junk DNA,” a “TDNA,” interrupting the sequence of TAF4b. This process hypothetically produces a generation of *Arabidopsis* plants that are unable to make a functional TAF4b protein due to the discontinuous genomic

sequence. When an organism fails to produce a particular protein product for this reason, it is said to be “mRNA null.” The degree to which an organism is mRNA null will have varying functional implications, and organisms who still produce an altered or truncated RNA transcript of the mutated gene will often manifest in an altered phenotype.

Before confirming whether my SAIL TAF4b-2 mutants were mRNA nulls, it became clear that even homozygous plants remained fertile, producing healthy seeds. This result was striking because it immediately suggested two interpretations: (a) that the *Arabidopsis* TAF4b homolog was not essential for gametogenesis and GSC maintenance as observed in mice, and/or (b) the SAIL mutant seed line did not contain mutation sufficient for disrupting the gene’s function. Upon initial characterization of the SAIL plants, neither homozygous nor heterozygous plants seemed to have seedling defects, reduced seed production, or altered floral organ structure. In a later count I found that only heterozygous TAF4b-2 mutant plants manifested in reduced seed set, and at a statistically significant level. When I discussed this result with members of the Pollen Genetics Lab, they suggested that reduced seed set was likely correlated with poor plant health, non-optimal watering conditions, or physical stress- an artifact of uncontrolled experimental conditions and not a phenotype. A second round of seed counts are planned to verify this result.

**Figure 15:** Result of seed counts, ANOVA for the results of heterozygous/WT and heterozygous/homozygous comparisons  $P= 2 \times 10^{-6}$ . A Tukey HSD test confirmed this result. The number of plants per genotype, followed by total siliques per genotype: N= 6 WT (blue) control plants and 60 total siliques, N= 5 TAF4b  $-/-$  (green) homozygous plants and total of 50 siliques, and finally, N= 15 TAF4b  $+/-$  (yellow) heterozygous plants and 150 total siliques,



In contrast, the TAF4b null male mouse develops an obvious and experimentally reproducible phenotype: complete lack of germ cells or “missing generations” of the progenitor cell population within weeks of birth (though notably not during fetal development of the germline) (Lovasco et al.). Additionally, the TAF4b mutation causes a severe ovarian aging phenotype in female mice, leading to a complete depletion of oogonia shortly after birth. Dr. Freiman and his students’ investigations revealed that TAF4b expression in the germline stem cells and surrounding embryonic gonadal tissues was likely crucial for normal mammalian neonatal germline development (Lovasco et al.; Falender et al.). Further, in their 2014 publication, Lovasco et al. find evidence strongly implicating TAF4b in the establishment and maintenance of mouse spermatogonial stem cells and gonocyte development as early as embryonic day 18.5. They concluded that “TAF4b-deficient spermatogonial progenitor cells” in their mutant mice “display a tendency for differentiation at the expense of self-renewal” (Lovasco et al.).

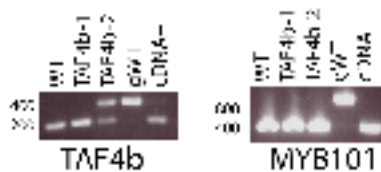
How did putative TAF4b null *Arabidopsis* compare to these mutant mice? Could the comparatively plastic stem cell fate of plants relative to metazoans be regulated by a homologous transcription factor in a similar manner? Given that the TAF4b-2 knockout lacked a discernable phenotype, we could not infer why TAF4b was only expressed in *Arabidopsis* pollen (and not ovules) (Schmid et al.), or why its mutation via TDNA mutagenesis did not seem to effect gametogenesis. Though TAF4b was crucial to the development and maintenance of mouse GSCs, its role in a plants, including *Arabidopsis*, remained unclear.

Significantly, the *Arabidopsis* TAF4b homolog studied in the context of this report has not appeared in the literature in any form. Why is this? My initial

characterization of the SAIL TAF4b-2 mutant plants suggests one possible explanation: the appearance of phenotypically normal fertility, and thus a lack of easily tractable phenotype. In our reverse genetic screens fertility alone was initially treated as (near) conclusive evidence of the expendability of TAF4b in plant gametogenesis.

Amidst seed counts, rtPCT (reverse transcriptase polymerase chain reaction) results failed to clarify the phenotype of the TAF4b mutant plants. RtPCR allows experimentalists to determine if a gene is being expressed by isolating the messenger RNA, reverse transcribing it into what is known as complementary DNA or cDNA, and then analyzing the cDNA products through gel electrophoresis. My experiments failed to definitively characterize the TAF4b mutant, even after three subsequent rounds of rtPCR involving the optimization of over four pairs of primers, small complementary strands of DNA that act to bind and isolate the gene of interest. In the context of experiment two, rtPCR was intended to determine whether or not the mutation resulted in an “RNA null” *Arabidopsis* plant. If rtPCR revealed no mRNA expression, we could conclude that the mutant plants were not able to express TAF4b. In fact, the results of my various rtPCR reactions were inconclusive, indicating that samples were contaminated with residual genomic DNA and possibly contained a reduced amount or truncated version of TAF4b mRNA. As noted earlier, the degree to which an organism is “RNA null” will have varying functional implications. Putative mutants that still produce an altered or truncated mRNA transcript will often manifest in an altered phenotype. To this end, Freiman confided, “it’s a dirty secret, but there’s really no such thing as an RNA null” mouse (Freiman and Johnson, “Initial Planning Meeting for TAF4b Pilot”). What both he and Dr. Johnson instead suggested was that I plan to design primers that “tile up and down”

the TAF4b mRNA sequence (Freiman and Johnson, “Winter TAF4b Meeting, PGL and Freiman Labs”), looking for truncations or pieces of what may or may not be missing from the TDNA-interrupted TAF4b transcript. The following figure depicts the products of one rtPCR reaction including samples from the mutant plant of interest (TAF4b-2) and various controls. The particular segment of the TAF4b gene represented here is “upstream” of the TDNA insert (site of mutation). Because primer pairs directly flanking the TDNA insert were not successfully optimized and the rtPCR result in Figure 15 is inconclusive, it remains unknown whether the TAF4b-2 mutant plants are truly mRNA nulls.



**Figure 16:** rtPCR result, five samples from left to right include WT (Columbia), TAF4b-1 (a SALK mutant seed line), TAF4b-2 (SAIL mutant seed line), WT genomic DNA control, and WT cDNA positive control; Bands in left block are the result of reaction with a 5' TAF4b primer set: Forward: TCCATTGTCAAGCTCCTCGA Reverse: GTCTTTTCCACCCAAGGAGTC Bands in the right block are a positive control, depict expression of MYB101, a ubiquitous transcription factor

## Stem Cell Populations

The assessment of TAF4b-mutant phenotypes and rtPCR results brought to light persistent differences in Dr. Johnson and Dr. Freiman’s operating definition of “stemness” and “germline.” First: do plants even possess stem cells? As mentioned earlier, many plant biologists assert that *Arabidopsis* has only two committed lineages of stem cell, shoot apical meristem and root apical meristem (Schmidt, Schmid, and Grossniklaus). While meeting with Drs. Freiman and Johnson, we reviewed a chart depicting the development of the inflorescence (floral) meristem. As is typical this figure characterized development in discreet, numbered stages (Cardarelli and Cecchetti).

MJ: So, point to where the germline initiates.



SW: Hmm. Tough question, uh, I mean, between stages five and six? Or earlier?

MJ: So these little bumps here, are floral meristems. Is that right?

SW: Yeah, I guess so.

MJ: So, this is going to make a flower.

SW: So would you say that the germline initiates there?

MJ: Maybe...um...now, comparing it to an animal, can you, at that stage, find cells that are the germ line?

SW: Uh- good question?

MJ: Uh, NO. But, are there cells there that after many more cell divisions will produce the germline? Yes.

(Freiman and Johnson, “Winter TAF4b Meeting, PGL and Freiman Labs”)

Defining discreet stages in the dynamic process of microsporogenesis is a matter of semantics as much as it is a comprehensive evaluation of developmental processes. When a long lineage of indeterminate cell precursors is demonstrated to be experimentally capable of giving rise to new life through regeneration, asexual reproduction, OR production of a haploid gamete for sex, what lines are drawn to define the contours of “germline?” In the case of *Arabidopsis*, Johnson notes that “stage six flowers” are not the germline but after many subsequent cell divisions could *produce* the germ line. In essence, this framing is consistent with Solana’s “PriSC” theory, which insists on germline continuity even in basal metazoans like planarian, whose fragmented body parts contain “neoblast cells” that maintain the capacity to give rise to entirely new bodies. Why are the germlines of similarly regenerative organisms with equally plastic stem cell fate invested with differential preformationist-type value?

SW: So, in like, laser ablation have people ablated very early inflorescence meristem cells?

MJ: Alright, so that's a great idea. So, is there a cell, or cells, that if you ablate it at that stage you would get a perfectly normal flower, except it wouldn't have germ cells? That's what you want, right?

SW: Sure.

MJ: There's no such cell.

SW: Alright.

MJ: So, does that mean that it doesn't have a germline at that stage? By this definition, no it doesn't. So in animals, you can do that, right? So like, urchins. You can ablate a cell and get a totally normal urchin but it just won't have a germline.

(Freiman and Johnson, "Winter TAF4b Meeting, PGL and Freiman Labs")

Using the hypothetical case of cell ablation and lineage tracing, we discussed how removal of specific spatially stratified cells within the developing inflorescence meristem would affect gametogenesis. Through this logic, Dr. Johnson arrives at a definition: in the absence of a direct precursor cell or lineage of primordial germline stem cells, no germline exists. Thus, hundreds of years of experimental precedent support his assertion that plants cannot have a phenotypically normal body composed of exclusively somatic tissue but lacking germline through cell ablation.

SW: No gametes.

MJ: And, uh... And in mouse you can do that right?

RF: But the fact that you have an inflorescence meristem, argues that there's a precursor-

MJ: Yeah, those are precursors-

RF: ...so...?

MJ: -there's no cell at that stage that is a dedicated precursor.

RF: Uhuh.

MJ: But there are precursors.

RF: Because we're taught that there's a shoot apical meristem and a root apical meristem. Right? But then- all of a sudden- there's an inflorescence?

MJ: So, right, so the shoot apex, at 'puberty' transitions to become an inflorescence.

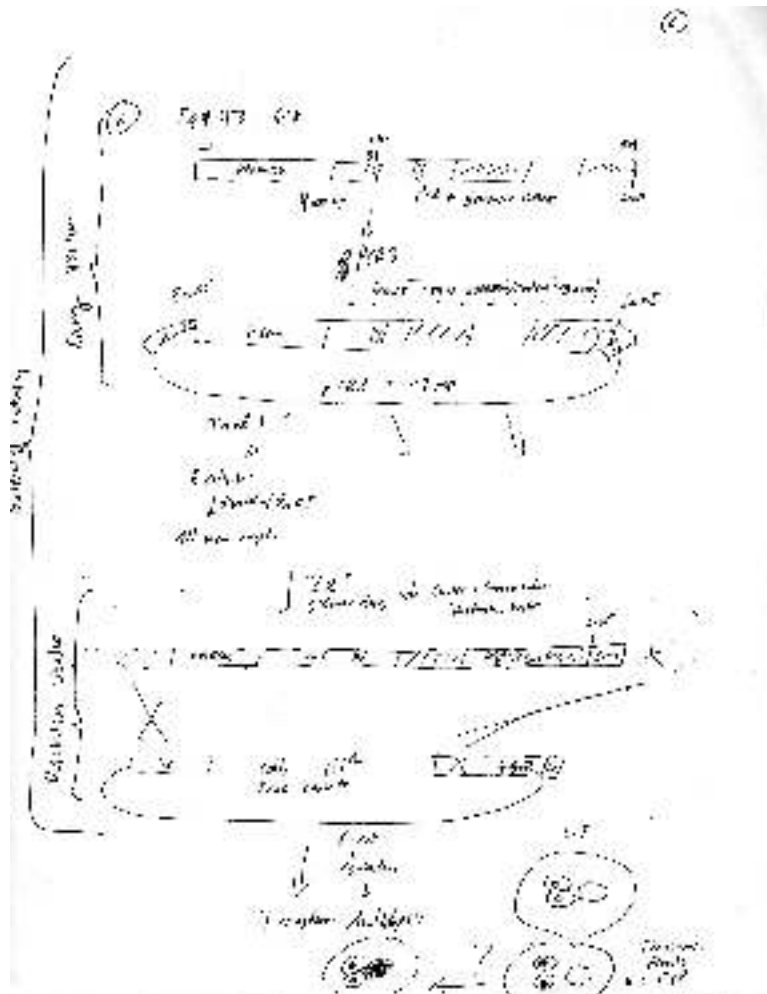
JF: At puberty?! [all laugh]

(Freiman and Johnson, "Winter TAF4b Meeting, PGL and Freiman Labs")

Finally, as often was the case in both PGL and Freiman labs, humor became the common denominator. Though Johnson's comment suggesting that *Arabidopsis* shoots go through "puberty" was meant to be facetious, it reveals 21<sup>st</sup> century conventions of developmental biology. Experimental results and a broadening literature review convinced me that, in some respects, my initial query into TAF4b *Arabidopsis* homolog was as outlandish as embarking on an investigating into plant "puberty." Why did the later notion provoke laughter while the former a tacit invitation to engage with laboratory study? This speaks to the fact that a time when scientific epistemologies allowed for "plant puberty" and overtly anthropomorphized accounts of reproduction are fading from memory. In contrast, the rationalization of gametogenesis through molecular, genetic, or biochemical evidence, once equally unimaginable, is normalized.

Do all sexually reproducing organisms have a distinct germline stem cell population? Is a germline constitutive of organs, cells, or sub-cellular macromolecules- the combined interactions of a gene regulatory network? Dr. Gary Wessel countered this question with the assertion that the germline is a “question of development, not a final state” (Wessel). Similarly, Anne Fausto Sterling argues that the reproductive capacities of “organisms-human or otherwise,” are developmentally “active processes, moving targets from fertilization until death (Fausto-Sterling). These re-articulations of reproduction demonstrate how germline stem cells -once imagined to be stable, distinct entities- are now increasingly characterized by malleable terms that originate across a diversity of species and local laboratory contexts. This dynamic condition enables GSCs to be adapted for a variety of biopolitical purposes, many of which increasingly destabilize the relationships between sex, gender and reproduction (Haraway, “A Manifesto for Cyborgs”). As demonstrated in the previous chapter, several newly “discovered” or popularized models and modes of inquiry into the germline unsettle boundaries between reproduction and regeneration, soma and germline, sex and syngamy. Similarly, because the plant and mammalian germline are expected to perform a variety of different tasks, the specific conditions in which their homology breaks down or remains comparable reveal instabilities in the definition of gametogenesis.

## IV. Transgenic Futures of the Germline and Stem Cells



**Figure 17:** Dr. Johnson sketches out experiment three, the creation of a transgenic *Arabidopsis* line, both entry vector and destination vector are shown, and at bottom right the hypothetical locations of TAF4b:GFP expression in pollen are shaded, specifically the sperm nuclei and vegetative nucleus of pollen

“...scientific and cultural objects of knowledge which have appeared historically since the Second World War prepare us to notice some important inadequacies in feminist analysis which has proceeded as if the organic, hierarchical dualisms ordering discourse in 'the West' since Aristotle still ruled...The dichotomies between mind and body, animal and human, organism and machine, public and private, nature and culture, men and women, primitive and civilized are all in question ideologically. The actual situation of women is their integration/exploitation into a world system of production/reproduction...One important route for reconstructing socialist-feminist politics is through theory and practice addressed to the social relations of science and technology...The cyborg is a kind of disassembled and reassembled, postmodern collective and personal self.” (Haraway, *Cyborgs*)

Experiments one and two interrogate the nature of evolutionary and historically produced differences in germ cell lineage commitment, sex and reproduction. In this final chapter I speculate about whether the germline exists within organisms, cells, or macromolecules, and how genome engineering, cancer and stem cell biology act to blur categories of health and disease, organism and cell colony. Experiment three involves the creation of a genetically modified plant for the explicit purpose of visualizing TAF4b expression. The comparison of Arabidopsis and mouse TAF4b homologs in this experiment exemplifies how molecular analogies of the germline are born, evolve through experimentation, and become adapted to fit new and different scientific paradigms. This experiment also provides a lens into how the germline can be manipulated and visualized through synthetic means, hinting at the profound effects of synthetic biology, stem cell engineering, and speculative biomedicine in remaking germline stem cells in the 21<sup>st</sup> century.

Of primary interest relative to the theoretical analysis of this chapter is the question of value: why does the study of human fertility create such a perceptible shift in the attitudes and rhetoric of the research community? Second, what does this reflect about the influence of biomedicine in producing the germline? And, finally, how does this exchange typify the fundamental inability of molecular biologists to define the scope of experimental questions; distinguishing between, “does this process happen endogenously and naturally in the body?” and “can this process be engineered to completion through genetic modification?” Germline stem cells of both male and female bodies are proliferating with the increase in defining features and classification schemes. Finally, though the scope of germline stem cell research is expanding to include non-classical

models, metaphors of human reproductive potential persistently structure theories of reproduction.

A humorous demonstration of this biomedical bias involves the presentation given by a student in Dr. Freiman's Stem Cell Biology class. The student's background in plant biology contrasted sharply with the majority of premedical students in class: "So, as you can tell from my title, I'm *not* going to be talking about humans, or stem cell biology in humans, or anything *related* to stem cell therapy in humans" (Draycott). This student's frustrations through a semester of anthropocentric descriptions of stem cell biology materialized in that moment and typify how a metazoan and biomedical bias acts across the field of stem cell biology, flattening organismal identity to enable biomedical translatability in research. As Johnson noted, plants don't have a germline stem cell population as defined by "animal people" (Freiman and Johnson, "Winter TAF4b Meeting, PGL and Freiman Labs"). 21<sup>st</sup> century molecular biology privileges the complex network of genes that regulate "stemness" and often must ignore the organism and its sex to enable useful molecular analogies across species and sex. As long as the information produced by germline stem cells proves useful in furthering speculative biomedical and biotechnical products, macromolecular descriptions of the germline stem cell population will prevail over species level definitions.

### **Visualizing TAF4b Expression in *Arabidopsis***

As my reverse genetic screens of TAF4b-2 mutant plants progressed Jen and I constructed a "full genomic TAF4b:GFP fusion." The purpose of this effort was to enable us to visualize TAF4b protein within *Arabidopsis*, a follow up to the potentially nebulous

results of reverse genetic screens in experiment two. This process involved creating a population of genetically modified *Arabidopsis* plants that express TAF4b protein with an attached green fluorescent protein tag (abbreviated TAF4b:GFP). Through this genetic manipulation, we hoped to make TAF4b expression visible in *Arabidopsis* pollen, and in doing so relate its form and function to the mammalian homolog. Historical precedent exists for a visual, histological definition of the germline stem cell population, though genetic manipulations as a means to this end were not developed until the turn of the 19<sup>th</sup> century.

After the emergence of cell theory in the 17<sup>th</sup> century, improved microscopy and dissection techniques allowed for new forms of GSC visualization. Before the invention of basic microscopy, the germline was not necessarily understood to occur exclusively within individual units like cells. Further, conceptualization of bodies as a collection (or colony) of cells became a prerequisite for understanding gametogenesis (Margulis, *Origins of Sex*). Technological improvements changed the mode by which biologists were able to visualize, and through their vision, give life to germline stem cells. 19<sup>th</sup> century techniques relied exclusively on dissection, staining, and keen observation of germline stem cells to distinguish what many scientists termed “nuage.” Nuage is something biologists consider to be a highly (evolutionary) conserved cellular feature characterized by “granular,” “electron dense...cytoplasmic material,” concurrent with a relatively large nucleus and single large nucleolus (Extavour and Akam). Additional factors included the observations of “bivalens,” first characterized by Boveri and Hertwig around 1890, fused chromosome tetrads resulting from the initial crossover phase preceding meiosis and marking the beginning of gametogenesis (Farley). These features



have long served as established scientific convention and are generally reconfirmed by 21<sup>st</sup> century molecular methods (Extavour and Akam) as reliable markers of germline “stem cell-ness.”

Despite historical precedent techniques common before the 21<sup>st</sup> century that rely exclusively on simple stains and microscopy (i.e. histology) are falling out of favor. When asked about relying on nuage as a positive and universal identifier of the germline, Dr. Wessel responded, “No, I would not be completely confident pointing to pieces of an egg [and saying this is a definitive biomarker of the germline]” (Wessel). 21<sup>st</sup> century tools that make germline stem cells visible to investigators like Wessel are primarily biochemical, involving the staining of molecular markers through immunohistochemistry or through measures of gene expression and epigenetic modifications tracked across developmental time points. In contrast, Dr. Boekelheide suggested that histological characterizations of germline stem cell identity, particularly in the mammalian testis, remain “the gold standard” (Boekelheide). In an era where the particular cellular biomarkers of pluripotency and stemness remain hotly debated (J. Johnson et al.; Tilly and Johnson; White et al.), there is a proliferation in the characteristics ascribed to the germline stem cell population relative to 19<sup>th</sup> and 20<sup>th</sup> century definitions.

In the case of TAF4b:GFP expression, we hoped to visualize the gene’s expression pattern in pollen or possibly earlier developmental stages of the male gametophyte. It was assumed that TAF4b:GFP expression would provide a reliable genetic marker for cross-species comparison of germline regulation and gametogenesis. Through this genetic manipulation, the analogous processes involved in mammalian and plant transcriptional regulation of the germline could be visualized and compared.

Despite great asymmetry in the tissue architecture, developmental processes, and gametogenesis of plants and animals at the organismal scale molecular biologists increasingly develop accounts of “conserved gene regulatory networks,” “pluripotency modules,” and “germline multipotency programs” to enable comparisons across species (Juliano, Swartz, and Wessel; Ewen-Campen, Schwager, and Extavour).

As discussed at length in chapter three, a persistent question in the biology of GSCs is: when does the germ line become separate from the soma? Correspondingly, a central question we hoped to answer using the TAF4b:GFP construct was the timing of TAF4b expressed in plant development, and more specifically, how this might illuminate genetically conserved function in establishment of the germline across diverse taxa like plants and mammals. Based on “microarray” data on the AtGenExpress Visualization Tool data database (referenced in the preamble), we expected to observe TAF4b:GFP expression in the sperm of tricellular pollen and potentially as early as the nuclei of free microspore precursors (See Figure 12, on page 62). As developmental biologists “strive to put biological processes into an evolutionary perspective,” Ewen-Campbell et al. suggest that the most pressing task becomes elucidation of “not just germ cell-specific genes,” but the “molecular interactions” of these gene regulatory networks in establishing germline identity (Ewen-Campen, Schwager, and Extavour). Further, the creation of this transgenic plant gave insight into larger narratives of germline stem cell identity emerging in the increasingly speculative realms of biotechnology and biomedicine.

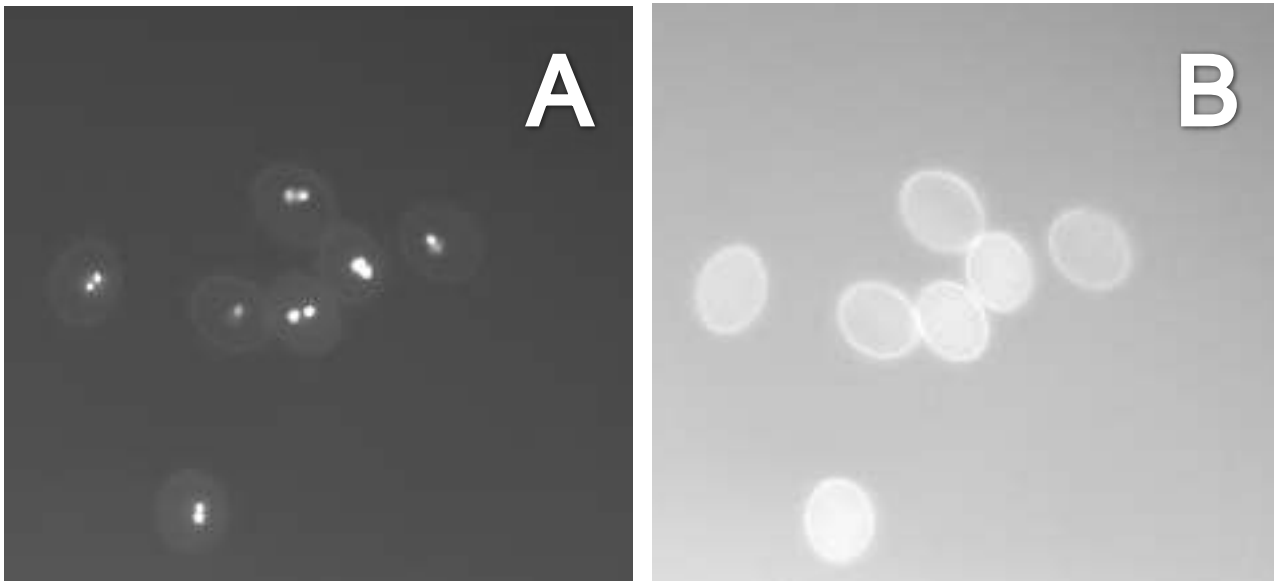
The process of genome engineering occurs at an ever-more rapid pace, though in the case of “TAF4b full genomic fusion” constructs, Jen and I worked for nine months to complete this process. Initially, the gene of interest is amplified through PCR, extracted

physically from the electrophoresis gel, and then cloned in the presence of an “entry vector.” Bacterial cloning happens “naturally,” in that *E. coli* happily “slurp up” spare DNA sequences in their environment, replicate the sequence through bacterial plasmids, then divide and begin again. Entry vectors are highly engineered circular bacterial plasmids- DNA- that contain genes enabling sequence specific recombination and antibiotic resistance. A second “destination vector” includes the GFP sequence and gene specific sites for integration of the cloned gene of interest. Once complete, the plasmid contains a total of three entities: an experimentalists’ gene of interest, a flanking GFP coding sequence, and an antibacterial resistance gene. Thus engineered, our plasmid is put “in culture” with *Agrobacterium*, which subsequently “take up” the GFP construct. Finally, *Arabidopsis* plants are dipped into a bacterial-laden concoction in which each bacterial clones each contain one identical TAF4b:GFP:Drug resistance plasmid. These plants are dipped when flowers are in bloom to ensure that the gametes, pollen and egg, are easily accessible to bacteria (thus enabling integration of the engineered sequence directly into the plant genome- for more background see appendix).

The result of this process initially appeared successful. The construct, or artificially created genetic sequence, contained the entire *Arabidopsis* TAF4b sequence, a gene for antibacterial resistance, plus its promoter region and the GFP coding sequence. Sequencing of the construct in Brown University’s genomic core facility revealed a single SNP, or single nucleotide polymorphism within the cloned region of TAF4b sequence. This SNP fell within a non coding, or intronic, region and was not considered to be a likely source of for problems downstream involving the expression of the TAF4b:GFP protein. Thus, when the progeny of transfected parent plants (the F1

generation) grew to maturity, I began eagerly preparing slides with pollen growth media for “screening” under the Zeiss Axiovert Fluorescence Microscope.

Despite our best efforts, the TAF4b:GFP full genomic fusion experiment did not give immediate insight into transcriptional regulation of the plant germline. Screening a total of 72 individual plants under the fluorescence microscope, including both germinated and ungerminated pollen, revealed no GFP “signal” (B). In no plant was a GFP signal visible above the background of pollen’s strong “autofluorescence” (B). The images below show pollen from a plant with HTR10:RFP marker transfected with our TAF4b:GFP construct. The HTR10:RFP marker is “sperm-nuclei specific” and results in RFP expression localized within the haploid sperm (A).



**Figure 18:** Fluorescent microscopy reveals the sperm nuclei in *Arabidopsis* pollen, a sperm nuclei specific HTR10:RFP signal (A); signal from GFP:TAF4b fusion not visible above autofluorescence in pollen (B), Scale: diameter of pollen grains is approximately 15uM

The fact that bacterial transformation during genetic engineering necessitates conjugation, horizontal gene transfer, and yet remains invisible in critical theory of

reproduction speaks to its force as a structuring paradigm. By Lynn Margulis's definition of sex, prokaryotes are in fact the one and only "cell," and thus constitutive of their entire germline (Freiman and Johnson, "Winter TAF4b Meeting, PGL and Freiman Labs"). How do geneticists differentiate between the bacterial body and the bacterial germline given their unicellular status? In conversation with Drs. Johnson and Freiman, Rich suggested that in the case of a conjugating unicellular organism, "it *is* a 'germ cell- it *couldn't have* somatic cells" (Freiman and Johnson, "Winter TAF4b Meeting, PGL and Freiman Labs"). Critically, bacterial "sex" enables the fluidity between "bacterial germline" and the Arabidopsis germline after transfection that is absolutely essential for creation of transgenic plants. In this way, ancient subcellular mechanisms of bacterial "sex" are coopted by biotech and silently but profoundly altering the norms and genetics of germlines everywhere. Where does the Arabidopsis "germline" start and the bacterial germline begin in my transgenic TAF4b:GFP expressing plants?

Does germline exist at the level of tissues, cells, or genetic sequence? The mingling of bodies in this experiment, necessitating both human and non-human actors, contradicts the simplistic notion of dichotomies between nature and culture, cell colony and organism (Haraway, "Speculative Fabulations for Technoculture's Generations"; Haraway, "A Manifesto for Cyborgs"). Explicit ties between developmental biology, stem cell engineering and biomedical therapeutics adapt anthropocentric conventions of the GSCs to the degree that it are useful and profitable. Despite durable biomedical bias, scientific discourses involving germline stem cells in mammalian, plant, and emergent *in vitro* cell culture systems are increasingly diverse.

### **Life *in Vitro*: Linking Transgenics, Development and Cancer of the Germline**

By the late 20<sup>th</sup> and early 21<sup>st</sup> centuries, *in vitro* cell culture techniques gave birth to a period of intense dispute and “fraud,” what Freiman jokingly referred to as “the wild wild west” of stem cell science (Freiman, “NANOG, Brown University Undergraduate Course in Stem Cell Biology”). This could also be interpreted as reflective of larger shifts in scientific paradigm, “revolutions” in how biologists understood the limits and scale of cellular life and natural or synthetic bodies (Kuhn; Helmreich, “Species of Biocapital”; Franklin; Bharadwaj). *In vitro* cell culture forced both biologists and laymen to reconsider the relation of cellular parts relative to the organismic whole. Additionally, the coupling of *in vitro* fertilization clinics with embryonic stem cell research primed American publics for culture wars around the defining moments of biological personhood (Franklin; Bharadwaj). Was an embryo a person? Who in society was charged with enforcing this definition, and how did speculative biocapital, an increasing movement to develop and commercialize and emergent biological research, act to privilege or marginalize specific accounts of fertility, germline, and genesis (Cussins)? Biomedicine dreams and fears the unlimited pluripotency of stem cells, and constructs many of its moral boundaries around perceived humane and human limits to the potential of such cells when cultured *in vitro*.

The molecular tools of cell culture allow humans to manipulate and control the germline to an unprecedented degree. The rise of *in vitro* cell culture profoundly effected definitions of “germ line” and “stem cell,” catalyzing a proliferation in the number of molecular variables biologists sought to control and describe. The precision and efficiency of *in vitro* cell culture and the removal of germline cells from their *in vivo*

(organismal, bodily, native) environment beginning around 1910 quickly proved to be a generative and industrious format for scientific knowledge production (Harrison). A reliance on “immortalized” (usually metazoan) cell lines, cells endowed with an indefinite ability to replicate induced by carcinogenesis or artificial (transgenic) means, expands operating definitions of “stemness.” 21<sup>st</sup> century knowledge about cell and molecular biology is increasingly dictated by cell colonies existing outside the boundaries of cohesive organic bodies, abstracted from organismal systems and *in vivo* bodily functions.

In one sense stem cell engineering has been remodeling the germline since Gurdon’s somatic cell nuclear transfer experiments of the 1970s. By enucleating the eggs of frogs and then transferring the nuclei of a fully differentiated, diploid keratinocyte back into the enucleated eggs, he notes that the experiment aimed to “find out whether nuclei undergo irreversible changes as cells specialize” (Gurdon et al.). Confounding notions of individuality and bodily limits, this landmark study also demonstrates the conceptual instabilities inherent to “nuage” and “germplasm.” Finding that his diploid “re-nucleated” frog eggs gave rise to normal embryos, Gurdon’s experiment fundamentally changed the fields of developmental biology, genetics, and reproductive biology, eventually earning him the Nobel Prize.

The Nobel prize was late in coming: awarded in 2012 concurrently with Shinya Yamanaka, a Japanese scientist whose research team succeeded in reprogramming mouse fibroblast cells to attain an “induced pluripotent” state (Takahashi et al.). Similarly, this publication enacted enormous and durable change in the field of stem cell biology. The realization that *in vitro* stem cell engineering could provide a means for “speculative

fabulations” (Haraway, “Speculative Fabulations for Technoculture’s Generations”), a seemingly blank slate on which the desires and biomedical needs of humanity could be reimagined, was critical. Thanks to a cascade of biomedical applications, questions of where, when, and how a human body becomes sexualized are in flux. Stem cell biology and *in vitro* fertilization proffered hopes that their “double reproductive value” could provide both regenerative medicine and fertility treatment for the masses (Franklin). These biopolitics also led to fear and mistrust of biotechnology, evidence by the Vatican’s condemnation of IVF as the “scientific creation of a baby” (“CatholicHerald.co.uk, Pope Francis”) and growing tension in communications between lay person and research institutions (Parry).

In 1977 Holden et al. suggested that the restricted expression of a particular cell surface marker protein “to embryonic and germ cells” indicated that this protein likely supported an “important developmental role” (Holden et al.). Thus, the explicit linking of cancer with regenerative, pluripotent function was catalyzed. Characterization of embryonic stem cell lines, called “embryonic germ cells” by Gail Martin and her colleagues in the 80s developed consensus around the transcriptional machinery of pluripotency (G. R. Martin). Martin’s melding of the terms, embryonic stem cell and germ cell into “embryonic germ cell,” hint at indivisible notions of pluripotency and stemness evolving in that time. Many of the transcriptional regulators characterized by Martin and in the wake of early embryonic stem cell science are included in Extavour and Wessel’s conceptions of a “conserved germ line multipotency program” (Juliano, Swartz, and Wessel; Ewen-Campen, Schwager, and Extavour).



This landscape fostered an unlikely fission between cancer, stem cell, and developmental biology centered around the characterization of “ancestral pluripotency modules” and dedifferentiated germ cell tumor phenotypes (Ewen-Campen, Schwager, and Extavour; Bustamante-Marín, Garness, and Capel). For example, Bustamante-Marín et al. note that testicular tumors “may hold the key to understanding how pluripotency is regulated *in vivo*” (Bustamante-Marín, Garness, and Capel). In their 2013 publication “Testicular Teratomas: an intersection of pluripotency, differentiation and cancer biology,” Bustamante-Marín et al. suggest that a scientific understanding of “teratoma susceptibility” has origins in “molecular understanding” of germ cell development. The language of germline cancer biology equates directly with the goals Freiman’s lab: to determine the “molecular etiology of fertility” (“Researchers at Brown: Richard N. Freiman Associate Professor of Medical Science”). Teratomas are pluripotent, embryonic stem cell-like cancer cells that can give rise to any of the three major embryonic cell lineages. Studies of teratomas through the lens of the germline or *visa versa* are not completed using *Arabidopsis* models. Despite this fact, the allegiance of cancer biology and developmental biology during the 70s acted to make fluid the boundary of “germline.” Shared accounts of teratomas and germline stem cells relied on the identification of shared molecular motifs associated with “stemness” and the germline, increasingly compressing these discreet cell identities into a definition delimited by gene networks and “pluripotency modules.”

The compelling tension invoked by conceptualizations of germline cells as both potent and pathological, gamete producing and cancerous, demonstrates a proliferation in the social tools used to define “germline” and “stem cell.” Bustamante-Marín et al. note

“the underlying pluripotency of germ cells during their migration and initial period of residence in the gonad (eg. Prior to E12.5 in mouse) represents a risk to the animal and is under tight regulation.” Conceptualized in the framework of risk, primordial germ line stem cells are defined by their molecular protein markers and gene regulatory networks, but understood to have potential tumorigenic tendencies. In the context of the Boekelheide lab toxic exposures to substances like DES, noted in the prologue, can perturb early germline gene regulatory networks and reconfigure the identity from germline cells into that of testicular teratoma.

### **The Egg and The Sperm: Narratives of a Gendered Germline**

Across centuries of scientific study, conventional gender roles are implicitly and explicitly deployed in the study of sexual reproduction (E. Martin, “The Egg and the Sperm”; Fausto-Sterling). Several accounts offer extensive proof that historically, biologically rationalized society and socially rationalized biology enable laboratory studies of plants and animals to naturalize male and female gender roles (Farley; E. Martin, “The Egg and the Sperm”; Fausto-Sterling; Bharadwaj). Critically, many of the experiments analyzed by critical theorists are focused on an explicitly biomedical analysis of behavior, development of genitalia, and hormones (Fausto-Sterling). Critical theory exclusive to the social-scientific origins of gender are less common at a cellular and molecular level (E. Martin, “The Egg and the Sperm”). In an era where entire departments are defined by the title “cell and molecular biology,” the gendering of cells, that is, the point when a cell is definitively understood to behave in a male or female mode, is a dynamic and increasingly nebulous enterprise.

A contemporary case study of how biochemical GSC identity, the expression of specific sets of molecular biomarkers like Oct4, has become a defining feature in the field involves the question of whether or not women possess a self-renewing population of oogonial stem cells post-natally. Deeply entrenched scientific conventions to this end had ruled that women do not possess such a population of cells (Boekelheide). In response, many theorists argue that socioscientific depictions of female fertility use language describing the process as inherently wasteful and necessarily limited in old age (E. Martin, “The Egg and the Sperm”). By this imagining of the human female germline, oogonial stem cells became characterized as limited and waning, in contrast to the male spermatogonial stem cells which were associated with inherent productivity and fertility. A counter-narrative emerged in 2005 with Joshua Johnson’s publication, “*Oocyte generation in adult mammalian ovaries by putative germ cells in bone marrow and peripheral blood*” (J. Johnson et al.). In this study, he and his colleagues postulate that an “extragonadal source of germ cells” sustains oogenesis in the mammalian ovary post-natally. Specifically, they argued that the hematopoietic stem cell population (HSCs) could circulate in the blood stream and thus reach their ultimate target, the ovary, and reestablish the female mammalian germline. Dr. Freiman assigned the various letters and publications related to this study as reading for his stem cell biology class, and noted that both sides of the debate had produced convincing evidence and he did not know what “side” to believe (Freiman, “SSCs, Brown University Undergraduate Course in Stem Cell Biology”).

This study caused a backlash and ongoing debate within the field (Tilly and Johnson; Eggan et al.; White et al.). Notably, Wessel described the research as “slimy”

due to reliance on layman's misplaced hope for an ultimate fertility therapy. Similarly Dr. Boekleheide suggested that Tilly was only seeking to be a "contrarian" and to "make a name for himself" (Wessel; Boekelheide). While some critical theorists of gender would welcome the Johnson et al. publication as a paradigm-altering approach to female reproductive biology, recasting ideas of potency, gender role, and aging processes, this would in fact oversimplify the speculative nature of the researchers' intent: developing biomedical fertility therapy to control and commercialize the germline in new ways.

In discussing this series of publications with Dr. Gary Wessel, he suggested that biomedical research aimed at managing human fertility is "game changing" (Wessel). Scientific debates about the existence of oogonial stem cells relate to fundamental questions of life because they involve a population of cells understood to have "unique reproductive potential," and are inherently value-laden because they come from the "context of [human] reproductive labor" (Keller and others). Biomedical therapy acts more or less explicitly in driving both the financial and theoretical processes of germline stem cell research. The formerly defined and limited potential of oogonial stem cells in this debate becomes nebulous and politicised, abstracted and eclipsed by the growth of an international market for speculative biomedicine and fertility therapies (Bharadwaj; Franklin).

In the Johnson et al. 2005 paper, "morphology, enclosure within follicles, and expression of germ-cell and oocyte-specific markers" are seen as definitive marks of a germline stem cell population (J. Johnson et al.) Critics argued that limited pluripotency "marker gene expression," i.e. *Oct4* expression, was not a definitive means for identifying oogonial stem cells. In a response letter, Tilly et al quote Carl Sagan, "Absence of

evidence is not evidence of absence” (Tilly and Johnson). Debate revolved around the legitimacy of Johnson’s determination of germline stem cell identity, and the necessity of arbitrary cutoffs for the type and number of biomarkers necessary to prove “stemness” and germline commitment. A varied geography of terms has sprung up to describe the inherently transitional state of stem cells both *in vitro* and *in vivo*. Thus, definitions of what characteristics define oogonial stem cells proliferated, introducing the potential for an origin in the hematopoietic stem cell population. These contradictory and competing explanations of GSC origin actively structure the field of reproductive biology, revealing just how unstable the definitions of GSC have become in 21<sup>st</sup> century biotechnology.

Particularly important in the study of the germline stem cells and gametogenesis is the development of speculative biomedical therapeutics are by coaxing fertile, haploid gametes from diploid, differentiated somatic precursor cells or *visa versa*. Stated more simply: a fertile haploid mammalian gamete can now be engineered from embryonic stem cell populations, and a mammalian spermatogonial stem cell can give rise to differentiated non-gametogenic cell populations. The *in vitro* culture experiments succeeded in manufacturing germ plasm and GSC identity in an environment entirely alienated from the somatic, gendered bodies. This research fundamentally alters the means and ends of gametogenesis, and places control of the germ line squarely in the hands of molecular biology. In 2006, Nayernia et al succeeded in producing haploid sperm-like cells that when fused with mouse ovules produced viable zygotes, capable of developing into full-term fetuses. They suggest that the transformation of these cells and sustenance in the absence of “somatic cell support” depends simply on a “cocktail of soluble growth factors, including retinoic acid (RA)” (Nayernia et al.). A related account,

Gallicano and Chen describe the “remarkable plasticity” of spermatagonial stem cells (SSCs), suggesting that they could be easily “reprogramed” for biomedical use (Gallicano and Chen). They emphasize the “great potential” for SSCs in developing “cell-based, autologous stem cell therapy,” and seek to characterize the molecular and genetic features underlying such plasticity. In discussing the definitions of “germ line” with Dr. Wessel, he initially noted that the germ line was a dynamic “developmental process,” and not solely the combine actions of a “gene regulatory network” (Wessel). Despite this fact, Wessel noted that the Nayernia et al experiment, provided salient evidence in favor of germ line definition as the combine actions of a “gene regulatory network;” damning proof against the continuity of the germplasm as speculated by Solana and Weismann.

Historical notions of the permanence and relative sanctity of a preformed and “set aside” germline are unraveling with the advent stem cell engineering; an era in which “gametogenesis” can occur “normally” *in vitro* (Nayernia et al.). Whether the simple addition of GFP “reporter genes” to track TAF4b expression in experiment three or the complete redirection of germlien stem cell differentiation in novel regenerative therapies as suggested by Gallicano et al., genetically modified organisms and cell lines are constantly redefining the terms germline and stem cell. Evelyn Fox Keller notes that “living organisms themselves are no longer the proper subject of biology” (Keller). Abstracted from the scale of bodies and gender altogether, the germline now thrives as concept and material object at the scale of cells and macromolecules.

## Conclusion



**Figure 19:** Arabidopsis seedlings used in experiments two and three, genotype noted on each tag

*“Sexual reproduction is one kind of reproductive strategy among many, with costs and benefits as a function of the system environment. Ideologies of sexual reproduction can no longer reasonably call on notions of sex and sex role as organic aspects in natural objects like organisms and families. Such reasoning will be unmasked as irrational, and ironically corporate executives reading Playboy and anti-porn radical feminists will make strange bedfellows in jointly unmasking the irrationalism.” (D Haraway, Cyborgs)*

Germline stem cells are born from a plurality model systems and increasingly unstable definitions. Their emergent lineage as concept and tool reflects larger social and political forces enabling biologists to understand the germline as a flexible, indeterminate population of cells with dynamic gene regulatory networks and manipulable developmental fates. Historical accounts of the germline and germline stem cells utilized categorical terms and exacting language, often calling on notions of sex and gender “as organic aspects” located in social and political bodies (Haraway, “A Manifesto for Cyborgs”). Instabilities in an what is imagined to be a unified definition of “germline stem cell” arise from 21<sup>st</sup> century questions of scale, redefinitions of sex and reproduction, and the emergence of speculative biotechnologies.

The establishment of massive, publically accessible gene data banks like NCBI’s “Genbank” ensure that study of the germline is centered around the “molecular etiology” of reproduction, a divergence from previous eras that privileged organismal, hormonal or organ system accounts of sexual reproduction (Keller; “Researchers at Brown: Richard N. Freiman Associate Professor of Medical Science”). This myopic focus on macromolecular machinery allows for species identity and organism to be abstracted, privileging networked biochemical, molecular, and genetic definitions of germ line. It is the approximations of these molecular networks by databases like GenBank that actively determine the material properties and limits of germline in real time, taking on a life of their own as an actor in the production of biological knowledge. Through such tools my own vision of the gene TAF4b in both *Arabidopsis* and mouse is filtered, consolidated, and contextualized, relating stemness, reproduction and sex at the macromolecular and cellular level.



Definitions of sex and reproduction proliferate with the characterization of “non-model organisms” and sheer complexity of gene regulatory networks. Though much STS scholarship provides salient evidence that “gender norms” act to organize the “mental and discursive maps of the social and natural worlds” (Keller and others), Haraway asserts that 21<sup>st</sup> century philosophies of reproduction “can no longer reasonably call on notions of sex and sex role as organic aspects” relative to “objects like organisms and families” (Haraway, “A Manifesto for Cyborgs”). Dynamic biological narratives of reproduction thus emerge in parallel with embodied social forms in society. The highly plastic germline program of plants and planarian, ability of parasexual organisms to undergo both sexual reproduction and asexual reproduction, and the recent production of fertile gametes through stem cell engineering all act to destabilize and decentralize scientific conventions of sex, gender, and reproduction. These processes are imagined by biologists not as materially distinct, but happening on a “continuum” enacted by dynamic cellular networks (Wessel).

The global biotech industry increasingly relies on germline stem cells and gametes, material and theoretical engines of biomedical production, for the establishment of both global and local scientific conventions. The manipulation, alteration, and control of these cell types in stem cell engineering enacts scientific conceptions of life and morality (Cussins). Despite the growing utility of “non-model” organisms, biomedical bias pervades the production of stem cell biology. This pervasive bias is rooted in deeply entrenched hope for the development of regenerative medicine technology and improved fertility therapies. The social technologies of stem cell “hope” provide dreams of immortality for the somatic and sexual body, a state of perfected biology free from the

natural limits of human sexual reproduction and illness. Stem cell technologies and their “double reproductive value” offer transcendence from exogenous and endogenous risk posed by disease, pollution, and aging (Franklin).

The germline stem cell population, a scientifically emergent and increasingly diverse lineage of cells, is in flux across species and models. Their identity as stem cell, conferring proliferative capacity and regenerative properties, and germ line, connoting the fecundity of gametes and potential for production of genetically novel life forms, is hybrid. Biological society privileges the property of “stemness,” the propensity of cells to proliferate and give rise to novel differentiated lineages, because its epistemic culture is organized around harnessing the material means of (re)production. Germline stem cells constitute a plethora of emergent forms, a lively linguistic tool that alternately allows for scientific determinism or vagueness as the situation requires. Correspondingly, the GSC population is alternately used to explain conventions of gender role, naturalize modes of reproduction in speculative biomedical therapeutics, and explain the etiology of cancer. Thus, various publics- scientific, biomedical or otherwise- require different forms and functions of the germline stem cell. Working definitions of “germline” and “stem cell” are consequently proliferating at an increasing rate. Ultimately, stem cells and gametes are thus contenders for the definitional power of reproduction, toiling for social recognition as the proper, capable, and natural entities that may give rise to life as society requires and defines it.

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