Communicating Research to the General Public

At the March 5, 2010 UW-Madison Chemistry Department Colloquium, Prof. Bassam Z. Shakhashiri, the director of the Wisconsin Initiative for Science Literacy (WISL), encouraged all UW-Madison chemistry Ph.D. candidates to include a chapter in their Ph.D. thesis communicating their research to non-specialists. The goal is to explain the candidate's scholarly research and its significance to a wider audience that includes family members, friends, civic groups, newspaper reporters, program officers at appropriate funding agencies, state legislators, and members of the U.S. Congress.

Nearly 100 Ph.D. degree recipients have successfully completed their theses and included such a chapter.

WISL encourages the inclusion of such chapters in all Ph.D. theses everywhere through the cooperation of Ph.D. candidates and their mentors. WISL is now offering additional awards of $250 for UW-Madison Ph.D. candidates in the sciences.

Wisconsin Initiative for Science Literacy

The dual mission of the Wisconsin Initiative for Science Literacy is to promote literacy in science, mathematics and technology among the general public and to attract future generations to careers in research, teaching and public service.

UW-Madison Department of Chemistry
1101 University Avenue
Madison, WI 53706-1396
Contact: Prof. Bassam Z. Shakhashiri
bassam@chem.wisc.edu
www.scifun.org

From niche signaling to its transcriptional control over *C. elegans* stem cells: an *in vivo* view

By

Tina Renee Lynch

A dissertation submitted in partial fulfillment of

the requirements for the degree of

Doctor of Philosophy

(Biochemistry)

At the

UNIVERSITY OF WISCONSIN – MADISON

2021

Date of final oral examination: 11/04/2021

The dissertation is approved by the following members of the Final Oral Committee:

Judith Kimble, Professor, Biochemistry
Catherine Fox, Professor, Biochemistry
Melissa Harrison, Associate Professor, Biomolecular Chemistry
David Wassarman, Professor, Genetics
Marvin Wickens, Professor, Biochemistry

4 Investigating the connection between DNA sequence and stem cell biology in a natural developmental context

4.1 Introduction: the molecular dance

4.1.1 Romanticism in biology

When I practice yoga, my favorite YouTube yogi, Fiji McAlpine, instructs me to imagine my breath moving through my body. Sometimes, I imagine my breath as some sort of abstract, purple-tinted wind moving down my arm, as if there were just empty space under my skin. Other times, I find it equally as meditative to imagine what "breath moving through my body" might really mean. I picture riding on oxygen molecules as they're sucked into air sacs in my lungs from the surrounding air and scooped up incredibly efficiently by hemoglobin proteins in my red blood cells. I imagine these oxygen passengers flowing through my bloodstream until they are drawn through the cell membranes into mitochondria organelles in the muscle tissue. There, the oxygen finally transforms to water as part of a biochemical cascade that harvests and stores energy for me to use in my next yoga pose. This kind of imagination is what drew me to science. To me, molecular biology and biochemistry are the "behind the scenes" of life on Earth. Yoga practice reconnects me with my sense of wonder that biological processes as apparently simple as breathing are driven by an endless probabilistic dance of blind and brainless molecular machines.

4.1.2 Why I wrote this chapter

When I arrived at grad school, I was lucky enough to join the lab of Dr. Judith Kimble. We study a biological process much more universal and even less conscious than breathing -- animal development.

It's convenient that I enjoy thinking about how biology is driven by the right number of molecules winding up in the right place at the right time – my project attempts to chip away at that very question as it relates to the development and function of the reproductive tissues that keep species alive through the millennia. I'm also lucky to be at the University of Wisconsin – Madison amongst a thriving community of people passionate about living up to the Wisconsin Idea. The Wisconsin Idea places university scientists as cogs in the societal machine, not above it. It asks what the use is of generating knowledge if that knowledge is not communicated to others who can use it to improve lives. Science helps produce food, medicine, and materials; it helps sustain democracy and innovation. To do all of this, newly generated knowledge needs to work for non-scientist specialists. We are responsible for reaching those specialists. We are also responsible for capturing imaginations in a broader swath of people than those who historically gravitated to science, because better science is in part fueled by drawing on imagination and unique personal experience. I'm grateful to be at a university where people like Professor Bassam Z. Shakhashiri recognized this and created programs like the Wisconsin Initiative for Science Literacy. I wrote this chapter for the taxpayers I work for, but also for me – the practice of science communication is not only enjoyable but helps my research advance.

4.2 Summary of thesis project

4.2.1 Why study worms?

Figure 4.1 *Caenorhabditis elegans* adult hermaphrodite at high magnification.
Actual size: about 1 millimeter long. Two fertilized eggs have just been laid. The translucent skin gives us a view of this tiny nematode's internal organs. Photo credit Maria Gallegos.

I'll be honest: before I started my PhD, I wasn't entirely sure that nematodes were real animals. Fortunately for me, the parasitic nematodes that live in intestines had no relevance to my life. I also don't have an agricultural background and still only dream of having a garden, so I hadn't deployed beneficial nematodes nor battled plant-infesting nematodes. Not only are nematodes real, they are the most abundant animals on Earth (van den Hoogen et al., 2019). Nematodes are a diverse phylum comprising more than 20,000 species of roundworms that span in size from fractions of a millimeter to feet long (Britannica). While parasitic nematodes are nightmare fuel, many other species are harmlessly free-living and are even called cute by some (me). Thankfully, the species I work with, *Caenorhabditis elegans*, is included in the tiny harmless group (Figure 4.1). So much can be gained from studying the nematodes themselves: in the ecosystem they can be prey or predator, pest or pest control (Lazarova et al., 2021; Schulenburg and Felix, 2017); they are useful in studying climate change not only for their role in nutrient cycling and carbon capture, but as an indicator of soil health (Mekonen et al., 2017); they can help us learn about how evolution shapes genetic diversity and impacts behavior (Crombie et al., 2019). It is clear that whether or not you are a nematode convert like me, whether or not you're aware of their relevance to your life, nematodes run our world.

My purpose for marveling at worldwide worm research is to draw a distinction between studying nematode ecology versus studying nematodes as a model organism. There is a ~120-year-long history of

scientists bringing nematodes, *C. elegans* in particular, into the laboratory to study fundamental biological processes, and I am part of that lineage (Nigon and Félix, 2018). From the late 1800s to mid-1900s, these worm scientists not only put in the hard work to figure out what foods and conditions kept the worms alive but studied cell division, sex determination, and ability to adapt to environment. The scientific relevance of the microscopic, translucent, free-living, cute nematode known as *C. elegans* is best underscored by the Nobel Prize in Physiology or Medicine of 2002. Sydney Brenner meticulously planned and demonstrated how *C. elegans* is supremely well-positioned to study developmental biology; John Sulston labored tirelessly watching embryos divide under a microscope to map the invariant path by which a single cell grows into an adult; Robert Horvitz put the work of the other two together to uncover a set of genes that regulate cell death as a crucial part of creating an adult from a single cell. Worms and humans share a lot of the same or similar genes, RNAs, and proteins (Kim et al., 2018). Thus, because the discoveries we make in the worms can often extend to human health or better understanding life on Earth, I don't say "I study worms" – I say, "I use worms to study stem cells."

Still, worms (especially to those outside the nematode convert community) seem like such an unsophisticated animal. In part, I have to agree – their body structure is essentially a tube within a tube. They don't even have a brain (just a nerve ring). They may rule the world, but they also occasionally wiggle up onto the walls of the plastic dishes we keep them in, get stuck, dry out, and die. However, they are powerfully tractable to study. Tractability means they are easy to keep, quick to grow, and that we have experimental options that produce readily interpretable results. With the support of Portal to the Public's STEM Fellows, I created an activity to demonstrate the tractability of worms. Imagine two jigsaw puzzles, identical in cut but not pattern (or, look at Figure 4.2). The *C. elegans* puzzle on the left is easier to solve than the human biology puzzle on the right because its color pattern is tractable – we can work with rainbow stripes, but it's much harder to work with a rainbow vomit where the colors don't even match up on the edges. In my actual science, the color pattern could represent the fact that the worms are

translucent and I can easily image the stem cell compartment I study. The key is that once we've understood more about *C. elegans* by piecing together the more tractable puzzle, it can offer hints that help us solve the more complex puzzles. In this case, there's a clue in the cut of the puzzles that narrows down the possibilities for solving the puzzle on the right (see figure legend). Thus, working in models like worms helps us accelerate discoveries that advance human health.

Figure 4.2 Puzzle activity to demonstrate model organism research.
Both puzzles are cut the same; they're made of the same pieces that are painted differently. On the left is the model organism puzzle – its tractability is represented by a color pattern that is easier to put together. Once the model organism puzzle is assembled, researchers can look for patterns that help them assemble the more complex puzzle on the right, which represents mammalian or human biology. In this case, the clue from the model organism puzzle that helps piece together the complex puzzle is that each row has pieces of the same shape.

4.2.2 My thesis project

Stem cells are originators: the rest of the cells in our bodies stem from them like branches from a tree trunk. The single-cell embryo can be thought of as the ultimate stem cell because it holds the potential to become any cell type in the body. As animal development progresses, other types of stem cells emerge. Early in development, embryonic stem cells can become most types of cells. Later in development, other more limited stem cells will produce specific tissues. In adults, tissue-specific adult stem cells are kept in

every tissue. They are tucked into special “niches” that protect and instruct the stem cells. Adult stem cells can generate more stem cells, or they can produce the specialized cells that directly participate in tissue function. The method of production is cell division, and each division might produce one stem cell and one specialized cell, or two of the same type. It’s important that stem cells balance their tasks of producing enough specialized cells to repair or maintain tissues and keeping enough stem cells to continue repairing and maintaining tissue over time. I study one type of adult stem cell: the germline stem cell. The germline is responsible for reproduction by producing the germ cells (sperm and egg cells) that pass on genetic information to the next generation.

The broadest phrasing of my research question is: how do species, generation after generation, faithfully produce offspring? Most of us are most familiar with one event that does help answer that question – the meeting of sperm and egg. But from a developmental biologist’s perspective, sperm meeting egg might be the end of the story. The full picture of offspring production is on a circular timeline (Figure 4.3). My research deals with the population of stem cells that produce the sperm and egg. These cells are represented with yellow in Figure 4.3. In the adult animal, shown at the top, the yellow stem cells are continuously producing eggs, which they fertilize with their own sperm (are you a nematode convert yet?!). At the bottom is the one-cell embryo – creating a reproductive system is so important that some cellular contents that will eventually become the germline stem cells are already localized to one side of the embryo. Worm larvae have a tiny germline that develops as they grow (left). The adults need to keep a population of germline stem cells at just the right size (back to the top). Too many stem cells, and the tissue is dysfunctional because it’s tumorous. Too few stem cells, and the tissue is dysfunctional because it can’t replenish or heal. Therefore, the very survival of not just individuals but the entire species rests on its ability to keep this population of cells at a Goldilocks level.

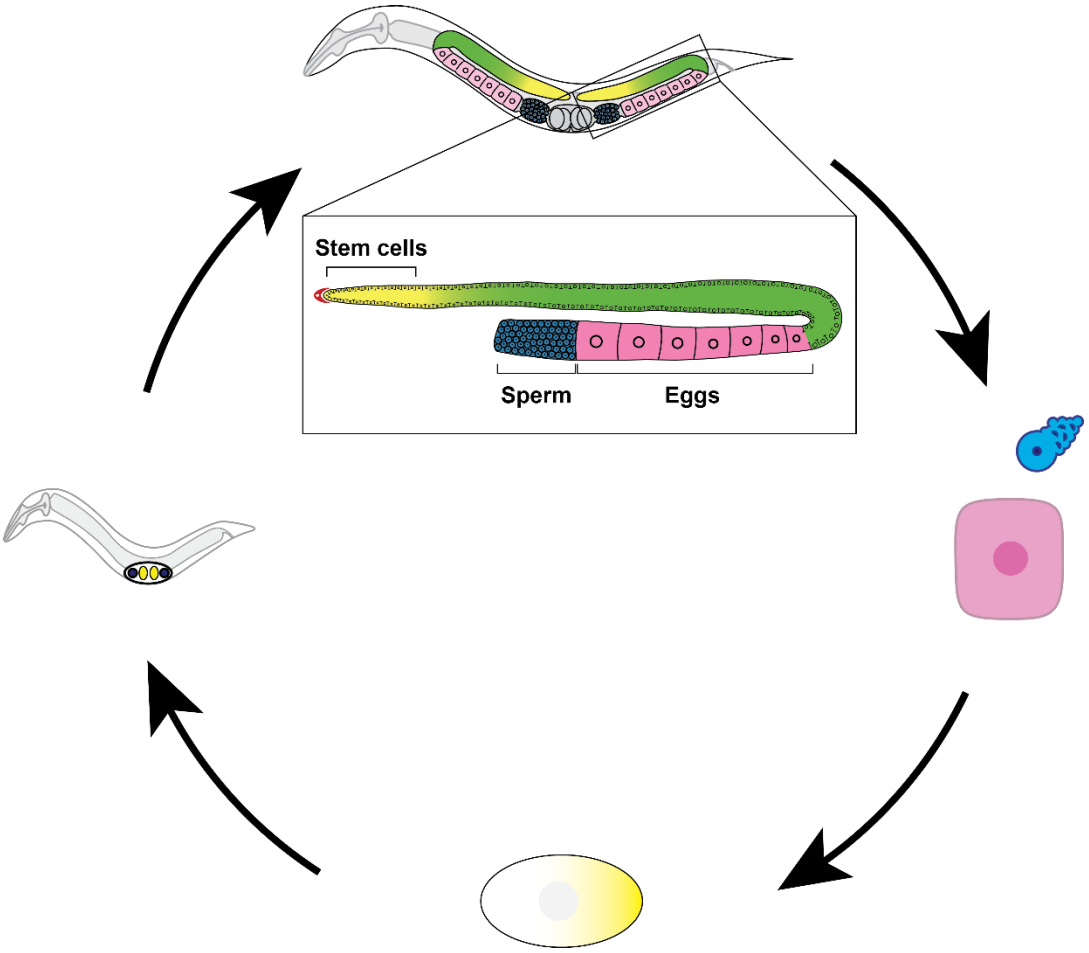

Figure 4.3 The unbroken chain of species survival.

Top, adult *C. elegans* with the germline tissue highlighted in color. Inset magnifies the germline tissue. Germline stem cells (yellow) live at the tip of the tissue. When they divide, they produce more yellow cells and/or green cells. The green cells eventually develop into either sperm (blue) or eggs (pink). I study how the animal regulates the size of the yellow stem cell pool. Right, sperm must meet egg to produce offspring. Bottom, a fertilized embryo, even from the one-cell stage, starts preparing this next generation's germline by localizing some mRNAs and proteins that give stem cells their special qualities over to one side. Left, newly hatched worms have four cells that will eventually divide to form the adult gonad.

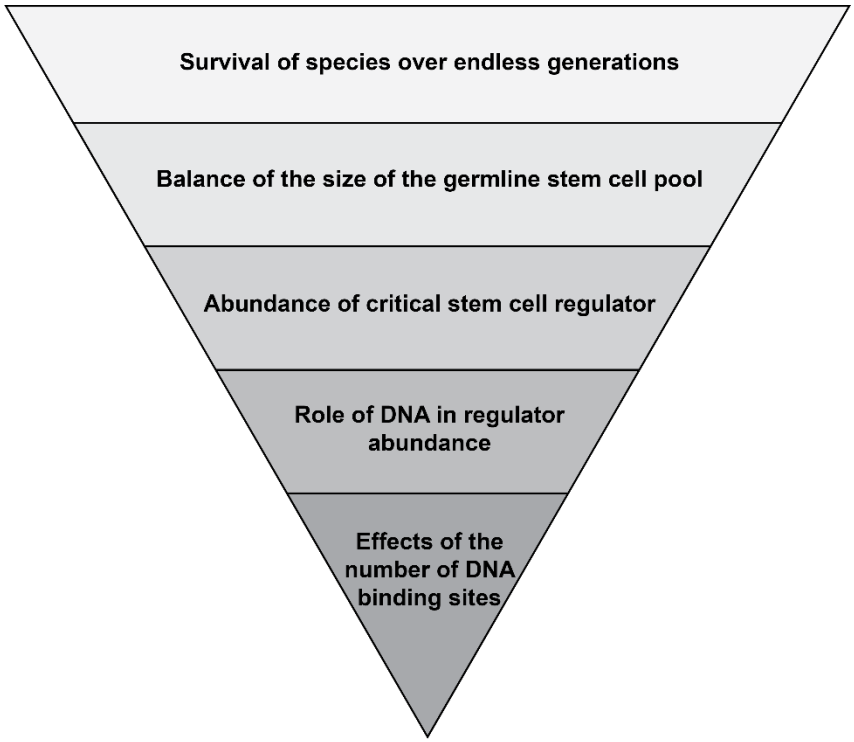

Figure 4.4 How do certain arrangements of DNA letters control the abundance of a critical stem cell regulator and therefore contribute to stem cell function?
Adult stem cell pools are in every tissue, and the number of adult stem cells must be balanced with the number of tissue-specialized cells to keep tissues healthy over the life of the animal. This general question is broadly applicable to the immortality of species, but also to health versus disease in individuals. In my specific project, the adult stem cell pool of interest is in the germline tissue, which makes sperm and eggs (see Fig 4.3). Ensuring that enough (but not too many!) critical stem cell regulator molecules are present in the right place at the right time is a major way to balance the stem cell pool size. My critical stem cell regulator of interest, a protein called SYGL-1, is controlled by specific arrangements of DNA letters (or bases); see Figure 4.5. My question is how specific arrangements of DNA bases contribute to producing SYGL-1 at a level that will help the stem cell pool stay balanced.

How groups of stem cells are kept at the proper size is a huge question that many scientists are studying through many angles and in many different contexts. I stay connected to that big question as often as I can, because the day-to-day questions we ask are much smaller and more tedious. “I’m studying the beautiful and mysterious phenomenon of species immortality” is the mantra that runs through my head when I drive to the lab on a weekend to feed worms, spend a long day in the frigid, dark microscope room, or pipette clear liquids into plastic tubes for the umpteenth time. Germline development cycles

over eons in an unbroken chain of events (Figure 4.3) and studying the stem cell pool size in adults is just one approach to learn how that happens (Figure 4.4). There are many mechanisms that act to balance the size of the stem cell pool, but I focused on one: the quantity and distribution of one protein called SYGL-1. I'm interested in SYGL-1 because it is a protein important for stem cell function. We know if there is more SYGL-1 than normal, the stem cell pool gets bigger. In fact, if there's too much SYGL-1, the whole germline tissue turns into a tumor of cells containing SYGL-1 (Shin et al., 2017). Again, there is more than one way the quantity and distribution of this SYGL-1 protein is determined, but I focused on one: the role of the DNA that controls SYGL-1 production (Figure 4.5).

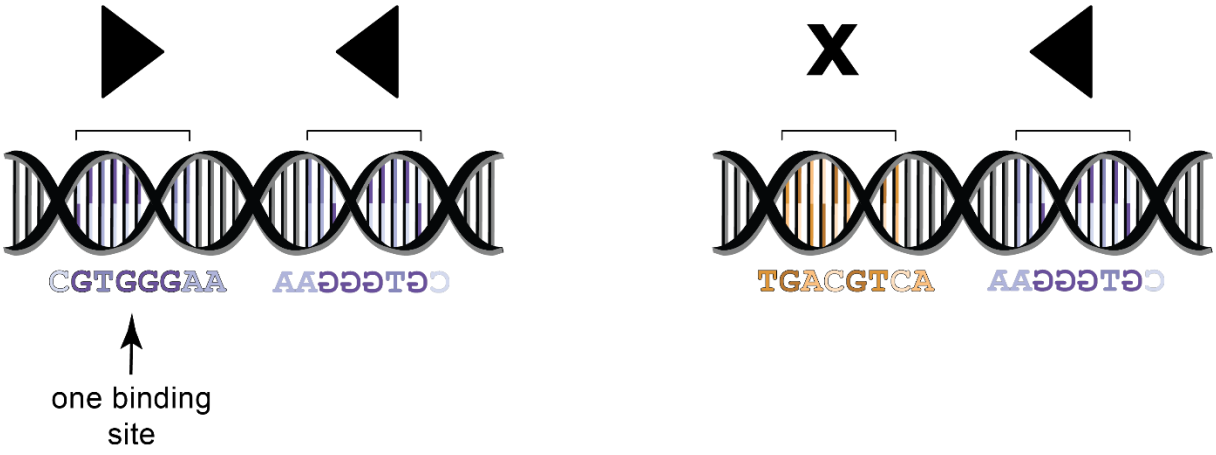

Figure 4.5 Mutating DNA binding sites in their natural context

Left, a stretch of wild-type (unaltered, found in nature) DNA. Right, a stretch of DNA with one binding site mutated (X) by CRISPR/Cas9 gene editing. Short arrangements of different DNA base pairs (in this case eight base pairs) constitute "binding sites" in the DNA. Bases are represented by color-coded letters (wild-type sites in purple, mutant site in orange): cytosine (C) lightest shade, guanine (G) darkest shade, thymine (T) second darkest shade, or adenine (A) second lightest shade. My binding site of interest has the sequence CGTGGGAA. Arrowheads above the binding sites are how I represent the binding sites when I zoom out from showing DNA structure as in Figure 4.6. The direction of the arrowhead indicates whether the binding site sequence is written facing upstream (CGTGGGAA) or downstream (its reverse complement, TTCCCACG). That combination of base pairs enables specific proteins to bind the DNA and initiate the production of RNA. RNA is converted to protein in the cell. My CGTGGGAA binding site of interest helps initiate production of *sygl-1* RNA, which is converted to SYGL-1 protein in the cell. I also studied *sygl-1* RNA abundance with a high-resolution technique that allowed me to count individual RNA molecules. I found that *sygl-1* RNA abundance correlates with SYGL-1 protein abundance and decided to tell this story focusing on SYGL-1 protein.

Once, a software developer friend asked me a charmingly engineer-minded question: if the problem is that we don't know which molecules are in the right place at the right time, then why can't we just build

super powerful microscopes and sit back and watch? I laughed and said that we would love to do just that, but there are two problems with that approach. The first is that our technology is not there yet and might never be. We have very powerful microscopes, but there are separate challenges to collecting live video data. The second problem is that observation is not enough -- we also need to identify the cellular components and test their functions. The beauty of science is often the elegance that comes from being forced to learn about the natural world indirectly. That indirect elegance has been particularly useful in the big-picture puzzle of how different arrangements of base pairs in the DNA direct the production of cellular components. DNA molecules are made of a backbone with 'rungs' that are a series of bases (represented by the letters A, T, C, and G). We still don't understand the full relationship between varied arrangements of base pairs in the genome and the correct number of molecules ending up in the right place at the right time. Much of what we know about the function of different DNA sequences comes from outside the DNA's natural cellular context, for example from test tube experiments. This is both because of technological limitations and because for some questions, answers are more interpretable when studied in a more controlled context. However, these elegant indirect approaches still leave caveats. Only studying DNA outside its natural context, we might eventually develop an in-depth rulebook for how a given stretch of DNA codes the production of 'x' amount of 'x' cellular component. However, we're amassing evidence that the context beyond the stretch of DNA itself (e.g. its neighborhood and neighbors in the cell nucleus) can be specific to cell type and change the way DNA directs production. Therefore, we might later find that a rulebook made with only one approach makes inaccurate predictions. Accuracy will be key when we are practicing precision medicine and developing stem cell therapies.

My contribution to this long-standing question was to directly study the relationship between DNA sequence and the production of the correct number of SYGL-1 molecules in *C. elegans*. I had access to a relatively new molecular tool, CRISPR/Cas9 gene editing, that allowed me to precisely change specific DNA bases in their native cellular context. One binding site is one group of DNA bases arranged into the

sequence CGTGGGAA. I identified six CGTGGGAA DNA binding sites, two far-apart clusters of three, that control the production of SYGL-1. I then mutated two or four of the sites and studied the effects of DNA mutation on the abundance of SYGL-1 and size of the stem cell pool (Figure 4.6). When I mutated two of six binding sites, leaving two per cluster, I measured about two thirds the normal SYGL-1 abundance and observed a shrunken but still functional stem cell pool. That amount of SYGL-1 supported tissue function, at least in cushy laboratory conditions where worms literally live inside a pile of food. My binding site mutants could be used as tools to explore whether tissue with reduced SYGL-1 might stand up to stress, because it would certainly experience stress in the rugged outdoors over eons. Individual animals also experience aging as stress. I next found that if I mutated four sites such that there was only one site per cluster, one isolated binding site could produce a tiny amount of SYGL-1, though not enough to support tissue function. By contrast, when I mutated four sites such that two binding sites were left close together in the same cluster, about one third the normal SYGL-1 was produced and tissue function was supported. These results are important because they advance our thinking about why DNA binding sites often occur in closely spaced clusters. My conclusion is that at least two neighboring DNA binding sites are needed to produce a sufficient abundance of SYGL-1 molecules in the stem cells to support adult egg production and a next generation of worms. That conclusion is free of the usual caveats that the effects we observe are not directly from the DNA mutation, or that effects may differ in the natural context.

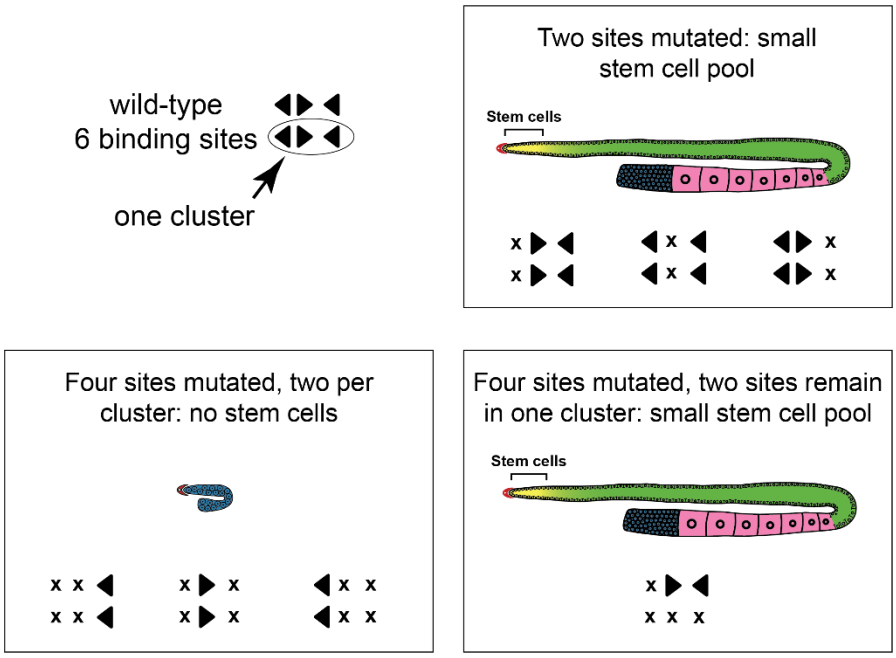

Figure 4.6 At least two neighboring DNA binding sites are required to support germline tissue function
Arrowheads represent DNA binding sites with a wild-type CGTGGGAA sequence and x's represent mutant TGACGTCA sequences, as in Figure 4.5. I found that the mutant sequence effectively destroys the ability of that binding site to produce *sygl-1* RNA or protein. By studying the effects of each possible combination of mutant and wild-type binding sequences (each square of six arrowheads and/or x's represents one population of nematodes' specific combination of binding sites). The binding sites are found in two clusters, which means one per chromosome (worms, like humans, have two copies of each gene).

4.3 Science is a process and a practice

4.3.1 A transformation: societal view to strong inference view

"Research is to see what everybody else has seen, and to think what nobody else has thought"

–Albert Szent-Gyorgyi

Albert Szent-Gyorgyi was a Nobel Laureate, an advocate for basic research, a highly praised teacher, and an anti-facist activist during World War II (NIH). His words inspire me. However, my interpretation of *why* this quote is inspirational has changed since I started my PhD. Years ago, my read of this quote

reflected my view of science as I had absorbed it from the culture I was steeped in: the best scientists are made from diamonds in the rough. They can "think what nobody else has thought" because they are inherently, exceptionally genius -- and they are rare. That idea is in the legend of Sir Isaac Newton and the apple tree: in the instant the falling fruit bonked him on the head, his theory of gravity was born – he had to rush off to write it down, presumably in its entirety.

But you might also have heard that Newton, Szent-Gyorgyi, Einstein, and other such now-renowned thinkers were judged too dim-witted to be scientists in their early lives. So what did they do to become greats? My next view of this quote was the message I absorbed from the academic culture: to see something different in what everyone else has seen requires totally obsessive devotion. You might be a diamond in the rough. Importantly, you might also just be rough all the way through – the way to expose potential brilliance, or to expose more dirt, is to polish hard. Sleep less, spend more waking hours obsessing about your scientific question, and one moment when you least expect it, your metaphorical apple will bonk the answer into your head too. Some do seem to get their apple bonk that way. For me, working so hard to prove to myself and others that I was inherently smart enough to succeed in science was far more effective at producing self-doubt, unhealthiness, and misery than producing better research. Judging by the rates of depression in academics (Satinsky et al., 2021), I don't think I'm alone.

My view has shifted again, and now I think the problem is searching for diamonds in the first place. Diamonds are formed with extreme heat and pressure, so that metaphor assumes individuals don't have the ability to create their own diamond – it's inherently there or not. I think the process is more like sculpting. There are myriad sculpting materials, sculpting tools, reasons for making a statue in the first place. Each scientist performs research by starting with a rough idea and refining over time. To see what everyone has seen and think what no one else has thought becomes two people who see the same hunk of marble and envision different sculptures that no one else has ever seen before. It is common (and encouraged!) to start sculpting with one vision in mind and end up sculpting something else. What joins

us scientists together is not that we're all aspiring Michelangelos, but that we're all guided by the scientific process. We're all sculpting very different things with different tools, but there are core principles of sculpting that anyone can learn, practice, and improve every day.

4.3.2 The scientific process of strong inference

I admit that I was a little harsh with the apple metaphor above – I didn't mean to tear down the usefulness of transformative moments in storytelling, just to emphasize how it seems too tightly wrapped up into the narrative of how science gets made. In fact, I'll use the apple bonk metaphor now. It's not that interesting for me to detail all the lessons from mentors, troubleshooting with my peers, gaining skills from colleagues I met through the UW Writing Center, reading, practicing, and reflecting that developed my view about the process of science. However, one moment does stand out as particularly transformative. My PhD mentor called a lab meeting to read and discuss two articles: The 1964 article 'Strong Inference' by J.R. Platt, and its 2014 reflection by Douglas Fudge. 'Strong Inference' describes a process that contains the same components I think many of us are familiar with: Observe, Question, Hypothesize, Predict, Test, Analyze & Conclude. This lab meeting was transformative because it made clear to me that this familiar series of steps is not really the core principle of science. The key is how the steps in the process are applied and practiced. I'm so grateful for this exceptional mentorship from Judith and Marv, their leadership by example that we graduate students are not just here to carry out experiments but to be trained as scientists in our own right.

Platt's thesis is that science is more efficient at uncovering knowledge when the scientist is systematic in their approach. His point is that simply following the steps of the scientific method will uncover knowledge much more slowly than if the scientist follows a few key rules. The hard parts of science, the parts we each need to always practice and improve, are what we choose to observe, how we approach the question, how many hypotheses we juggle, and that the conclusion matches the analysis. One rule to

vastly improve your scientific practice, Platt writes, is to apply Francis Bacon's method of strong inference. Platt devises a useful strong inference test: "it consists of asking in your own mind, on hearing any scientific explanation or theory put forward, 'But sir, what experiment could *disprove* your hypothesis?'; or, on hearing a scientific experiment described, 'But sir, what hypothesis does your experiment *dis*prove?'" Of course, it's a mark of 1964 that Platt assumes each scientist calls themselves sir in their head. Funnily enough though, the voice in my head that asks me this question in 2021 certainly calls me sir, and also wears a feathered cap. The second rule is to follow geologist T.C. Chamberlin's advice to have multiple hypotheses. Platt points out that it is human nature to become emotionally attached to a single hypothesis that seems to explain the observation satisfactorily, and also human nature to avoid disproving hypotheses we are emotionally attached to. He quotes the philosopher Karl Popper: "there is no such thing as proof in science – because some later alternative explanation may be as good or better – so that science advances only by disproofs." Theories aren't even theories, Platt says, "unless [they] can be falsified by some possible experimental outcome." Otherwise, they "predict everything, and therefore [do] not predict anything."

Our lab discussion of the 1964 'Strong Inference' article was transformative because it gave me a well-defined framework to continually improve my practice of science. Our lab discussion of the 50-year anniversary revisit article was transformative because I finally started believing that I really could be a scientist. One take-home message from Fudge's article was that the Strong Inference process requires different skills for each step: sensitivity, curiosity, creativity, imagination, ingenuity, and logic. From that, our lab discussed how each individual scientist might have different blends of these skills. It was transformative to hear Judith, who knew some of my Nobel Laureate science heroes personally, tell stories about how even a hero might have creativity and imagination in such spades they're lacking some ingenuity and logic. As a group, we also discussed how teamwork is important to science because we can help balance each other out. Individual scientists don't need to be the best at every science skill to

succeed. Instead, we should each find our niche -- identify our own strengths and collaborate with people who have different ones. So much of the cultural picture of scientists is the lone genius tinkering alone in lab at midnight on Christmas Day. Part of that is truth – we are smart and do have an unfortunate habit of working through holidays. But that picture misses those same holiday tinkerers also swapping science stories and inspiring each other informally over a glass of wine. Returning to the tale of Newton, he was sitting alone under an apple tree and rushed off alone to record his thoughts. It turns out there really was an apple tree that caught Newton's attention – but it was after dinner with his friend William Stukeley (Stukeley, 1752). I'm speculating here, but I think that in contrast to a falling apple bonking ideas into his head, Newton said, "Hey Bill, you ever think about why apples fall straight down and not sideways?" William Stukeley, the 18th century physician and clergyman, probably replied something like, "Huh. Nope." They probably had a short and light conversation about it, and Stukeley probably asked a question that only a non-physicist would ask but that made Newton go, "I hadn't thought of it that way." Then, over the next weeks or months or years before publishing his theories, Newton kept taking out this idea with the apples and grappling with it.

I wrote this section on the process of science because, much like my results, these words are a direct product of my PhD research. Research is often difficult and slow and discouraging, and it makes most or all of us question whether we belong. Because I struggled with self-doubt seriously and regularly, a big part of my PhD experience was investigating some potential reasons, using that knowledge to build self-confidence, and trying to help others do the same. The flawed cultural narrative of what it takes to be a scientist is pervasive. Those who are minoritized by systemic societal biases are discouraged from pursuing or staying in higher STEM degrees at higher rates (Nicole and DeBoer, 2020). In my opinion, the cultural narrative and the way we tend to treat each other because of it contributes to those statistics. If recording my experience here could help fellow young scientists find or reinforce their belief that they belong, that's worth more to me personally than anything else in this document. Another reason is that

the scientific process is not just for science. I'll end on the words from Douglas Fudge that stuck with me most: "Platt suggests that making progress requires walking a razor's edge between audacity and humility, and applying each in the right place at the right time. It is a message that can benefit anyone who is interested in tackling difficult problems."

4.4 References

Britannica, T. E. o. E. Nematode.

Crombie, T. A., Zdraljevic, S., Cook, D. E., Tanny, R. E., Brady, S. C., Wang, Y., Evans, K. S., Hahnel, S., Lee, D., Rodriguez, B. C., et al. (2019). Deep sampling of Hawaiian Caenorhabditis elegans reveals high genetic diversity and admixture with global populations. *Elife* **8**.

Kim, W., Underwood, R. S., Greenwald, I. and Shaye, D. D. (2018). OrthoList 2: A New Comparative Genomic Analysis of Human and Caenorhabditis elegans Genes. *Genetics* **210**, 445-461.

Lazarova, S., Coyne, D., G. Rodríguez, M. G., Peteira, B. and Ciancio, A. (2021). Functional Diversity of Soil Nematodes in Relation to the Impact of Agriculture—A Review. *Diversity* **13**.

Mekonen, S., Petros, I., Hailemariam, M. and Conservation, B. (2017). The role of nematodes in the processes of soil ecology and their use as bioindicators. *Agric. Biol. JN Am* **8**, 132-140.

Nicole, F. and DeBoer, J. (2020). A Systematized Literature Review of The Factors that Predict the Retention of Racially Minoritized Students in STEM Graduate Degree Programs. In *2020 ASEE Virtual Annual Conference Content Access*.

Nigon, V. M. and Félix, M.-A. (2018). History of research on C. elegans and other free-living nematodes as model organisms. *WormBook: The Online Review of C. elegans Biology [Internet]*.

NIH The Albert Szent-Gyorgyi Papers.

Satinsky, E. N., Kimura, T., Kiang, M. V., Abebe, R., Cunningham, S., Lee, H., Lin, X., Liu, C. H., Rudan, I., Sen, S., et al. (2021). Systematic review and meta-analysis of depression, anxiety, and suicidal ideation among Ph.D. students. *Sci Rep* **11**, 14370.

Schulenburg, H. and Felix, M. A. (2017). The Natural Biotic Environment of Caenorhabditis elegans. *Genetics* **206**, 55-86.

Shin, H., Haupt, K. A., Kershner, A. M., Kroll-Conner, P., Wickens, M. and Kimble, J. (2017). SYGL-1 and LST-1 link niche signaling to PUF RNA repression for stem cell maintenance in Caenorhabditis elegans. *PLoS Genet* **13**, e1007121.

Stukeley, W. (1752). Memoirs of Sir Isaac Newton's Life.

van den Hoogen, J., Geisen, S., Routh, D., Ferris, H., Traunspurger, W., Wardle, D. A., de Goede, R. G. M., Adams, B. J., Ahmad, W., Andriuzzi, W. S., et al. (2019). Soil nematode abundance and functional group composition at a global scale. *Nature* **572**, 194-198.