Treasure Your Exceptions: An Interview with 2017 George Beadle Award Recipient Susan A. Gerbi

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THE Genetics Society of America's (GSA) George W. Beadle Award honors individuals who have made outstanding contributions to the community of genetics researchers and who exemplify the qualities of its namesake. The 2017 recipient is Susan A. Gerbi, who has been a prominent leader and advocate for the scientific community. In the course of her research on DNA replication, Gerbi helped develop the method of Replication Initiation Point (RIP) mapping to map replication origins at the nucleotide level, improving resolution by two orders of magnitude. RIP mapping also provides the basis for the now popular use of λ-exonuclease to enrich nascent DNA to map replication origins genome-wide. Gerbi's second area of research on ribosomal RNA revealed a conserved core secondary structure, as well as conserved nucleotide elements (CNEs). Some CNEs are universally conserved, while other CNEs are conserved in all eukaryotes but not in archaea or bacteria, suggesting a eukaryotic function. Intriguingly, the majority of the eukaryotic-specific CNEs line the tunnel of the large ribosomal subunit through which the nascent polypeptide exits. Gerbi has promoted the fly *Sciara coprophila* as a model organism ever since she used its enormous polytene chromosomes to help develop the method of *in situ* hybridization during her Ph.D. research in Joe Gall's laboratory. The Gerbi laboratory maintains the *Sciara* International Stock Center and manages its future, actively spreading *Sciara* stocks to other laboratories. Gerbi has also served in many leadership roles, working on issues of science policy, women in science, scientific training, and career preparation. This is an abridged version of the interview. The full interview is available on the Genes to Genomes blog, at genestogenomes.org/gerbi.

How did you get involved with the March for Science?

As scientists, we have an obligation to share with the public what our science is about. Of course, this has always been true, but it seems especially true in the current era. I was really spurred on by (GSA President) Lynn Cooley at the fly meeting, where she challenged me when she was presenting me with the Beadle Award. She mentioned that I had played a role in public policy through the American Society for Cell Biology and through the Federation of American Societies for Experimental Biology, as well as through the American Association of Medical Colleges. And then she said, "we need you now!" I went home and I thought: yes, the field needs people to be actively involved in public policy at this particular time in history. So, with some difficulty, I found the local leaders for the March for Science in Rhode Island, and then played an active role in mobilizing the Brown community.

Even though the March for Science itself was amazingly successful, it must go on beyond that. We need to speak to our congressional representatives, we need to speak to the general public, and to our neighbors about what we do, why it is exciting, and why it is important for advances in our society.

What inspired you to become a scientist?

My father was a physician scientist. When I was a youngster he would bring me to lectures at the New York Academy of Sciences, which was terribly exciting. I would be learning about things in high school biology and then would get to hear talks by the people making the discoveries. Holley spoke about the structure of tRNA, for example, and Palade about ribosomes, and Nirenberg about cracking the genetic code.

What drew you to studying chromosomes?

I became interested in chromosomes in high school after reading a Scientific American article by J. Herbert Taylor, who had discovered that the replication of chromosomes was semiconservative. Then when I went to Barnard College I had the opportunity

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to take a molecular genetics course with Herb Taylor, and that confirmed my interest in chromosomes and replication. I knew I wanted to do a Ph.D. on chromosomes, and one of the emerging leaders in the field at the time was Joe Gall. So, I applied to Yale.

It was a fortuitous time to be in his laboratory because the method of molecular hybridization had just emerged from the work of Spiegelman, where radiative probes are hybridized to DNA captured on nitrocellulose filters. It was a no-brainer to try to expand that to the chromosome level. Joe Gall went to a meeting in South America where several scientists brainstormed about how they might best apply this method. They all went home to their laboratories and got hung up on the controls. But Gall, being a fabulous biologist, said he was going to use a system where he knew what the biological answer *should* be and then he would work things out from there.

He and my fellow grad student Mary-Lou Pardue worked out the initial method of *in situ* hybridization. They used the stage of meiosis in *Xenopus* oocytes, where you find thousands of nucleoli that contain amplified ribosomal DNA. The next step was to apply the method to chromosomes themselves rather than amplified nucleoli, and I was part of that effort. We did the first *in situ* hybridization to chromosomes using the gigantic polytene chromosomes from the salivary glands of the lower dipteran *Sciara*, as well as *Drosophila*.

How did your long interest in ribosomes begin?

The probe we used in the *in situ* hybridizations was ribosomal RNA labeled with tritiated uridine, and we used *Xenopus* rRNA because it was available from tissue culture cells. I wondered how *Xenopus* RNA could hybridize to fly chromosomes. I thought there must be some sequences that have been retained during evolution, and that started me on the long path of studying eukaryotic ribosomal RNA using evolution as a guide.

We started with *Xenopus* rRNA because it was the first eukaryotic gene ever cloned. By hybridization, we found there were regions of conservation even between bacteria and eukaryotes. Then we produced our first rRNA sequence. We modeled it using principles of compensatory base changes—where base-pairing in hairpin stem regions would be retained even if the sequence changes—and we found that there was a core structure that was conserved between *Xenopus*, yeast, and *Escherichia coli*.

What can we learn from understanding *Sciara* rereplication?

DNA rereplication leading to gene amplification is a hallmark of many cancers, but the underlying mechanism is not fully understood. One cannot induce amplification in cells in a way that allows you to study the initiating events; you only see the final outcomes of amplification. So, it became very desirable to look for model systems where this is a natural part of development.

There are two known cases of developmentally-programmed locus-specific rereplication: *Drosophila* follicle cells and salivary gland polytene chromosomes from the end of *Sciara* larval life. We want to understand how these origins of replication bypass normal cellular controls.

What is the function of developmentally programmed rereplication?

The areas that undergo rereplication in the *Sciara* polytene chromosomes are called DNA puffs; they serve as templates for a massive amount of transcription that is translated into the proteins needed to make the pupal case in the next stage of development. In both *Sciara* late larvae and in *Drosophila* follicles there is a very short window in which a massive amount of protein is needed.

You might ask why other cell types do not use the same strategy. The problem is that once you have undergone rereplication, you now have nested replication forks and a structure called an onion-skin that is potentially very unstable; but in both *Sciara* polytene chromosomes and the polyploid cells of *Drosophila* follicle cells there is no mitosis, so the onion-skin structure is not damaging. In addition, both tissues are destined to be destroyed soon after the rereplication event, so they would not have to live with the consequences anyway. If such onion-skin structures occur in dividing cells—such as in the cells that become cancerous—this might lead to breakage and recombination, and eventually lead to amplification.

What have you learned about rereplication?

The first thing we had to do was understand what an origin of replication looks like at the sequence level. We developed a method that we called RIP mapping. This was done with Anja Bielinsky, who was a postdoc in my laboratory. We needed an enriched population of newly replicated DNA to start with, and for this we popularized the use of the enzyme λ-exonuclease. This will digest DNA from its 5′ end in an exonucleolytic fashion, but not if there is an RNA primer at the end, such as there is after rereplication.

Once we established where DNA synthesis starts in rereplication, we could look at the surrounding sequence. We found that adjacent to the start site there is a potential binding site for an ecdysone receptor. This is the master regulator of insect development, and it was the first transcription factor ever discovered. We are trying to test whether it is also acting as a replication factor. If so, the question is whether —in hormonally-sensitive cancers such as breast cancer—the estrogen receptor might also serve as an amplification factor.

You are a great advocate for Sciara. What is so compelling about this species?

Sciara is an amazing model organism with many unique biological features. Geneticists usually figure out how things work by making mutations. But, if you will, the unique features

in *Sciara* are like God-given mutations; they are variations of canonical processes that can shed light on the underlying mechanism.

In the 1930s, geneticists had a meeting at Cold Spring Harbor and realized that they would make more progress if they all worked on the same organism. They discussed which to choose, and the two finalists were *Sciara* and *Drosophila*. We all know who won! The reason *Drosophila* was chosen was because geneticists of the 1930s relied on making mutations by X-irradiation, and *Sciara* turns out to be extremely resistant to X-irradiation.

Fast-forward to the current time, and of course now we do not have to rely on X-irradiation for mutation. We have established a toolbox of the *Sciara* genome sequence and a methodology to transform *Sciara*, so the time is now ripe for the scientific community to study all the unique features of this species. We give a 1–2-day workshop in my laboratory for anyone who wants to learn how to work with *Sciara*.

What are some of the unique features of *Sciara*?

One is sex determination. There is no Y chromosome, and sex is determined by the mother. Something, possibly in the cytoplasm, is conditioned by the mother at an early stage prior to meiosis. Spermatogenesis is also unique. In the first meiotic division in males there is a monopolar spindle. What is remarkable is that in the anaphase-like configuration, all the paternally-derived homologs move toward the nonpolar end of the spindle. This was the first example of imprinting. It was noticed by Helen Crouse, who coined the term imprinting in her 1960 *GENETICS* paper.

So, all of the paternally derived homologs move away from the single pole and are then discarded. In a way, this is a system en route to parthenogenesis because—at least in sperm—it is not using the paternally-derived chromosomes of the previous generations. The chromosomes that move toward the single pole are maternally derived, and of course, how chromosomes move to this pole is a fascinating subject that is worthy of study in itself.

Then, in meiosis II a bipolar spindle is established. So now the chromosomes do align on a metaphase plate and then segregate, with the exception of the X. The X instead stays locked into the single centrosome, and the result is two products of meiosis II: one is nullo-X (which is discarded) and the other has two copies of X (the X dyad).

So, the only product of spermatogenesis is a single cell that has two copies of an X and is haploid for the autosomes. At fertilization, you have one X from the egg and two from the sperm, and the zygote ends up with three copies. But, of course, you cannot keep doing this every generation! So, in an early cleavage division some of the X chromosomes are eliminated.

If the offspring is going to be male, it eliminates one of the three Xs; if the offspring is going to be female, it eliminates two of the three Xs. Now imprinting comes into play. The eliminated Xs are always paternally derived. The X chromosomes that will be eliminated line up on the metaphase plate and start to separate, but the arms of the Xs fail to separate. So, it is as if there is a chromosome-specific failure of the cohesins to dissolve.

It turns out that there is a region that was genetically identified by Crouse that she called the controlling element (CE). It governs the X dyad nondisjunction in meiosis II, as well as the X chromosome elimination in embryogenesis. You can move the CE locus to any of the three autosomes by reciprocal translocations, and now you have fooled the cell into treating the autosomes as if they were the X. The CE is located within the tandem array of 50 copies of ribosomal RNA genes; it is right in the middle of the array and is flanked by translocation breakpoints. So, we would like to be able to zero in on it with long-read sequencing and terrific genome assemblies.

In addition to the sex determination mechanism and the unusual behaviors imparted by the CE, *Sciara* also has germline-limited chromosomes called the L chromosomes, whose roles are totally unknown. And, in addition, *Sciara* has locus-specific rereplication in DNA puffs of polytene chromosomes and other unique features.

What advice would you give to younger scientists?

Treasure your exceptions. *Sciara* is an exception to the way things normally happen, but it can give you an enormous amount of insight into the basic canonical mechanisms that are shared by most other organisms. If you get a result in the laboratory that is unexpected, do not throw up your hands in despair. You may in fact have opened up a whole new line of pursuit!