The Genetic Map Enters Its Second Century

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The Genetics Society of America (GSA) Medal is awarded to an individual for outstanding contributions to the field of genetics in the past 15 years. Recipients of the GSA Medal are recognized for elegant and highly meaningful contributions to modern genetics and exemplify the ingenuity of GSA members. The 2015 recipient is Steven Henikoff, whose achievements include major contributions to Drosophila genetics and epigenetics, Arabidopsis genetics and epigenetics, population and evolutionary genetics, genomic technologies, computational biology, and transcription and chromatin biology. Among these achievements, Henikoff elucidated the mechanism for position-effect variegation, revealed a central role for variant histones in nucleosome assembly at active genes, and provided new insights into genome evolution. He has also developed widely used computational tools for genome and protein analysis and new strategies for mapping chromatin-binding sites.

 Genetics was born with Gregor Mendel’s classic paper (Mendel 1866), but it was A. H. Sturtevant’s introduction of the first genetic map in 1913 that defined the field of genetics as we know it today (Sturtevant 1913). For several decades, the genetic map was an ordering of genes along the chromosome determined by crossing over and chromosomal rearrangement. Beginning in the 1970s, restriction endonucleases allowed for genetic mapping to be based directly on DNA, which was eventually supplanted by DNA sequencing. As we enter the second century of genetic mapping, we are witnessing a progression from a genetic map defined by DNA sequence to a map enriched by the epigenomic landscape. Here I describe this latest transformation of the genetic map from the perspective of an observer and participant.

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— S.H.

My interest in genetic maps began in the mid-1980s when I discovered a glaring exception to Sturtevant’s linear order of genes along the chromosome: a pair of “nested genes,” in which a Drosophila pupal cuticle protein is encoded within an intron of the Gart purine biosynthetic pathway gene encoded on the opposite strand (Henikoff et al. 1986). By then, traditional “forward” genetics based on a genetic map was giving way to “reverse” genetics, which begins with homology searching and ends with phenotypic analysis of a mutant. Much of our attention during the reverse-genetics era was focused on improving homology searches, leading to popular tools such as the BLOSUM series of amino acid substitution matrices (Henikoff and Henikoff 1992) and SIFT (Sorting Intolerant From Tolerant) by graduate student Pauline Ng for predicting deleterious substitutions (Ng and Henikoff 2001). Homology searching led to discovery of a novel Arabidopsis DNA methyltransferase (“chromomethylase”) (Henikoff and Comai 1998), along with a decade-long collaboration with Luca Comai and a thesis project for graduate student Claire McCallum. Confronted with the need to obtain chromomethylase mutants, Claire came up with the idea of screening PCR-amplified pools of mutagenized DNA for induced point mutations (McCallum et al. 2000). What we called “TILLING” (Targeting Induced Local Lesions IN Genomes) evolved into a reverse-genetic service for the Arabidopsis (Till et al. 2003), maize (Till et al. 2004), and Drosophila (Cooper et al. 2008) communities based on mismatch cleavage discovery of point mutations. Luca Comai and his colleagues have since developed TILLING into a sequencing-based strategy for efficient discovery and mapping of mutations and polymorphisms in crop plants (Tsai et al. 2011). TILLING for nontransgenic crop improvement also continues in the developing world, an effort spearheaded by former Seattle TILLING Project leader...
Bradley Till at the Food and Agricultural Organization of the United Nations.

In the late 1990s postdoc Bas van Steensel had the idea of creating genome-wide epigenetic maps using tethered Dam DNA methyltransferase to mark sites of DNA- and chromatin-binding proteins along the chromosome (van Steensel and Henikoff 2000; van Steensel et al. 2001). Later in his own lab, Bas developed this DamID technique into a tool for probing the large-scale structure of the nucleus, mapping, for example, the five “colors” of Drosophila chromatin (Filion et al. 2010). Meanwhile, our introduction of microarray-based DNA methylation profiling as a readout platform for DamID led to the discovery of gene-body methylation by postdocs Robert Tran and Daniel Zilberman (Tran et al. 2005). Daniel showed in his own lab at the University of California at Berkeley that this epigenomic feature is ancestral for eukaryotes (Zemach et al. 2010). As readout technologies advanced, epigenomic mapping flourished, including Solexa-based mapping of DNA methylation imprints in the Arabidopsis endosperm by postdoc Mary Gehring (Gehring et al. 2009).

Our most recent tool development efforts have been motivated by a long-term interest in understanding epigenetic phenomena. Postdoc Kami Ahmad’s discovery of the replication-independent pathway for nucleosome assembly that incorporates the H3.3 histone variant (Ahmad and Henikoff 2002) led graduate student Yoshiko Mito to introduce a biotin-tagging protocol for chromatin immunoprecipitation (ChiP) of H3.3-containing nucleosomes (Mito et al. 2005, 2007). Our genome-wide implementation of the classical salt-fractionation method indicated that the low-salt-soluble “active” chromatin fraction is enriched for the H2A.Z replication-independent histone variant and so might be turning over during transcription (Henikoff et al. 2009). To directly test this possibility, postdoc Roger Deal introduced the CATCH-IT (Covalent Attachment of Tags to Capture Histones and Identify Turnover) metabolic labeling method for measuring nucleosome turnover (Deal et al. 2010). Based on CATCH-IT we estimated that nucleosomes over epigenetic regulatory elements were turning over too fast for histone modifications themselves to be inherited between cell divisions. Graduate student Sheila Teves used CATCH-IT to show that transcription drives nucleosome turnover (Teves and Henikoff 2011), and postdoc Fan Yang used it to show that anthracycline chemotherapeutic drugs enhance transcription-coupled turnover (Yang et al. 2013) and double-strand breaks (Yang et al. 2015) around mouse promoters.

Roger Deal’s interest in applying high-resolution epigenomic tools to the study of Arabidopsis root development converged with Daniel Zilberman’s discovery that H2A.Z and DNA methylation are mutually exclusive in Arabidopsis (Zilberman et al. 2008), followed by evidence from graduate student Melissa Conerly that this is also the case for gene bodies in cancer cells (Conerly et al. 2010). Studies of Drosophila H2A.Z by graduate student Chris Weber showed that just downstream of the transcriptional start site nucleosomes are preferentially “homotypic,” containing two H2A.Z molecules (Weber et al. 2010), which suggested that disruption of nucleosomes by RNA Polymerase II (RNAPII) facilitates H2A.Z incorporation. Chris next asked how the collision of RNAPII with a nucleosome affects RNAPII transit, but he realized that published methods for RNAPII profiling in higher eukaryotes were inadequate. He reasoned that the extreme insolubility of RNAPII would allow recovery of its attached nascent transcript in the washed chromatin pellet, and so by sequencing the 3’ end, he could map all RNAPII species, including those that are invisible to run-on methods because they are stalled and backtracked. Indeed, Chris’ simple “3’NT” method allowed him to show that essentially all RNAPII stalls and backtracks when it encounters each nucleosome in its path (Weber et al. 2014). Analysis of 3’NT data also allowed Chris and postdoc Srinivas Ramachandran to infer the biological role of H2A.Z, namely to modulate the nucleosome barrier to transcription. Chris’ 3’NT method precisely maps the addition of each base to the growing RNA chain within the active site of RNAPII without the need for antibodies or tags, making it potentially suitable for many applications. Meanwhile, Sheila Teves devised a high-resolution protocol for mapping DNA torsion genome-wide (Teves and Henikoff 2014), which, together with CATCH-IT and 3’NT, provides a powerful set of mapping tools for elucidating transcriptional dynamics in vivo.

To take advantage of the potential for massively parallel short-read sequencing, Jorja Henikoff, graduate student Kristina Krassovsky, and I developed a simple modification of MNase-seq with fragment midpoint-by-length display to achieve base-pair resolution of the full chromatin landscape (Henikoff et al. 2011; Henikoff and Henikoff 2012; Krassovsky et al. 2012). This native chromatin became input for high-resolution ChiP-seq of nucleosome remodelers by postdoc Gabe Zentner (Zentner et al. 2013) and for transcription factors by graduate student Siva Kasinathan (Kasinathan et al. 2014).

Dr. Henikoff’s research has moved the entire field of genetics forward through a combination of technical innovations and fundamental discoveries. His selection as the recipient of the GSA Medal is a fitting honor to a scientist who inspires so many of us in so many different fields.

—Dan Gottschling, Fred Hutchinson Cancer Research Center
Meanwhile, postdoc Pete Skene developed a high-resolution cross-linking ChIP-seq protocol to map highly insoluble complexes (Skene et al. 2014).

The ultimate mapping challenge is the centromere, as evidenced by the fact that centromeres remain as multimegabase gaps in the current human genome assembly. My interest in centromeres and surrounding heterochromatin began with a suggestion from my postdoc mentor, Charles Laird, to look into the classical phenomenon of position-effect variegation, and our later interest in histone variant evolution led us to explore this final frontier of the genome. The chromatin foundations of centromeres are nucleosomes containing the cenH3 (CENP-A in mammals) histone variant, and beginning in 1999 Paul Talbert and others in the lab began to identify and characterize cenH3s from worms (Buchwitz et al. 1999), flies (Henikoff et al. 2000), Arabidopsis (Talbert et al. 2002), and rice (Nagaki et al. 2003). I was intrigued by the poor sequence conservation of cenH3s despite the near perfect conservation of canonical H3 and variant H3.3. Postdoc Harmit Malik discovered that the basis for divergence is positive selection, which was unprecedented for a protein that is essential for every cell division (Malik and Henikoff 2001) and led to the concept of “centromere drive” (Malik and Henikoff 2009). In his own lab at the Fred Hutchinson Cancer Research Center, Harmit Malik has gone on to test predictions of the centromere drive hypothesis, including possible mechanisms for centromere evolution and the origin of species (Bayes and Malik 2009), while efforts in my lab have focused on characterizing cenH3 nucleosomes.

Studies by postdocs Yamini Dalal and Takehito Furuyama led to a model for the cenH3 particle as a right-handed half-nucleosome (“hemisome”) (Dalal et al. 2007; Furuyama and Henikoff 2009), which we have confirmed in budding yeast using high-resolution native ChIP (Krassovsky et al. 2012), in vitro reconstitution (Furuyama et al. 2013), and in vivo chemical cleavage mapping (Henikoff et al. 2014). Meanwhile, postdoc Florian Steiner’s mapping of worm holocentromeres revealed them to be dispersed cenH3 single-wrap particles, each resembling a yeast “point” centromere (Steiner and Henikoff 2014), which suggests that worm holocentromeres are polycentric, an idea first proposed by Franz Schrader 80 years ago (Schrader 1935). Surprisingly, insect holocentromeres entirely lack cenH3, as shown by postdoc Anna Drinnenberg, who is jointly mentored with Harmit Malik (Drinnenberg et al. 2014).

Our recent progress in applying high-resolution mapping tools to centromeres of yeast, rice, and worms has encouraged us to analyze ChIP-seq data by de novo computational mapping to explore the tandemly repetitive α-satellite sequences in which human centromeres are embedded—our genome’s “black hole” (Henikoff 2002). Here we encountered another surprise: precisely phased single-wrap CENP-A particles occupying young α-satellite dimeric repeat units, in contrast to the diffusely phased CENP-A particles occupying older pericentric higher-order repeats (Henikoff et al. 2015). Our finding that two dimeric units dominate functional human centromeres and show decreasing abundance with evolutionary age provides a fascinating glimpse into the dynamic genetic process that continuously generates α-satellite. I am optimistic that we will soon be able to connect DNA sequence to centromere function in this final frontier of the genetic map.

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