

Greater Focus for Fuzzy Epigenomes

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Epigenetics describes heritable changes in gene expression mediated by factors like DNA methylation and histone modification, rather than by changes to underlying DNA sequences. Tumors exhibit major disruptions of histone modification and DNA methylation, making epigenetics one of the hottest current topics in cancer research. Ever since the genetic code embedded in DNA was cracked over half a century ago, tremendous progress has been made in assembling genome sequences for a number of species. In contrast, specification of the 'epigenome' – defined as the distribution of epigenetic factors across any given genome at any point in time – has lagged behind, owing largely to the highly dynamic nature of the epigenome and a lack of tools for quantifying its structural variation.

A breakthrough in the Basic Sciences Division, recently published by lead author Jorja Henikoff, graduate student Kristina Krassovsky, principal investigator Dr. Steven Henikoff and two outside collaborators, offers a new tool for viewing dynamic epigenome structure at single base-pair resolution. Their study focuses on mapping nucleosome- and subnucleosome-sized particles in yeast. Nucleosomes are the basic unit of DNA packaging, consisting of DNA wound around a core of eight histone monomers. Nucleosomal DNA 'breathes' in and out as it fluctuates between wrapped and unwrapped states, and nucleosomes slide along DNA under the influence of ATP-dependent nucleosome remodelers. The result is dynamic variation in DNA accessibility, which has big implications for transcription factor binding and DNA expression. The new approach of Henikoff *et al.* employs standard DNA digestion by micrococcal nuclease (MNase), which nibbles away faster at exposed DNA compared to DNA protected within nucleosomes or by subnucleosomal structures. The authors combined MNase digestion with a paired-end library preparation protocol, which they modified to efficiently recover tiny DNA fragments protected by DNA binding proteins while excluding primer and adapter sequences.

Mapping the millions of resulting reads against the yeast genome, Henikoff *et al.* revealed a much higher resolution picture of the positions of nucleosomes and subnucleosomal particles with respect to any gene of interest. They found that subnucleosomal particles occupy nearly all intergenic regions, and that these particles are enriched in nucleosome-depleted promoter regions. They suspected that many of these subnucleosomal particles are ATP-dependent remodelers, which promote eviction of neighboring nucleosomes at a high MNase concentration. Evidence of this was

provided specifically for the subnucleosomal particle residing over the Gal4 promoter region. The authors then developed a graphical approach to characterize the positions of nucleosomal and subnucleosomal particles by constructing ‘V-plots’ of read position *versus* length (see figure). When applied to known transcription factor binding sites, their ingenious plots can help illuminate functional relationships between native transcription factors and nucleosomes, including the phasing of nucleosomes the same distance away from either side of a transcription factor binding site. The Henikoff lab’s protocol for MNase mapping of native chromatin will allow researchers to undertake the epigenome equivalents of genome sequencing projects in any cell type or species.

[Henikoff JG, Belsky JA, Krassovsky K, MacAlpine DM, Henikoff S.](#) 2011. Epigenome characterization at single base-pair resolution. *Proc Natl Acad Sci USA* 108:18318-18323.

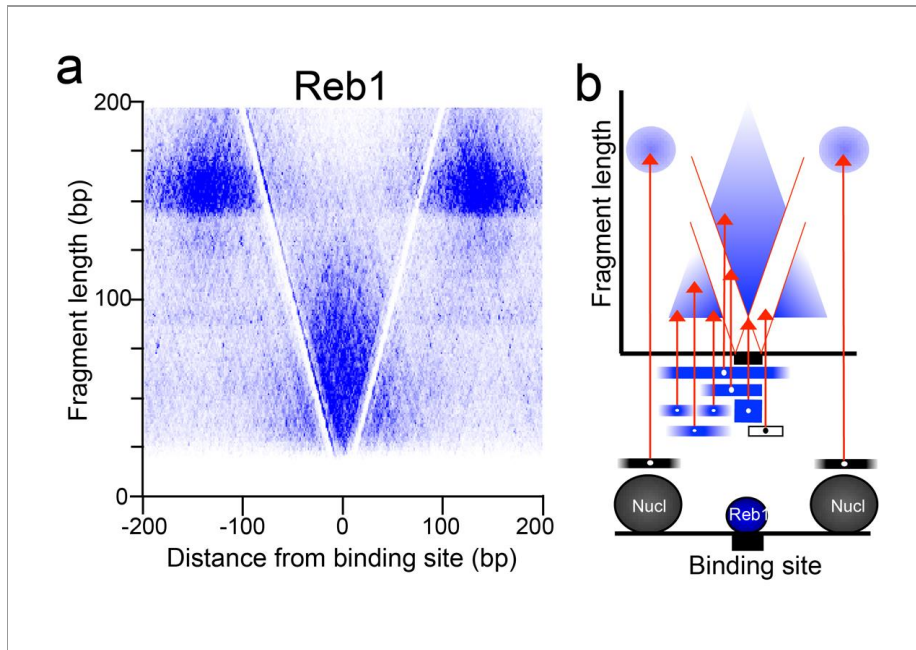


Image courtesy of Steven Henikoff

V-plot of a transcription factor and flanking nucleosomes. a) Experimentally determined Reb1 binding sites from throughout the budding yeast genome are aligned around the consensus Reb1 DNA-binding motif. A fragment midpoint versus length dotplot map (V-plot) of the 400-bp region spanning Reb1 transcription-factor binding sites is shown. b) Diagram illustrates how the map is interpreted. Fragments are indicated below the graph. A fragment that spans a protected region (first blue fragment) results in a dot placed in the central sector (vertical red arrow). The left diagonal results from fragments cleaved precisely on the right side of the protected region (second blue fragment), but at random on the left; likewise for the right diagonal. The minimal protected region is at the intersection of these diagonals on the y axis, and is also represented as the width of the gap on the x axis (extrapolation of the diagonal red lines to $y = 0$). The open box represents a fragment that is not observed because its left end is protected from cleavage. Flanking triangle densities are produced by dispersed protected fragments that are near or adjacent to Reb1 binding sites, but are cleaved in between.