Class 9: Analyzing chromosomal pretein binding sites using chromatin immunoprecipitation (ChIP)

Reading assignment

- Pokholok et al. (2006) Activated signal transduction kinases frequently occupy target genes. Science 3134:533-536
- Guillemette et al. (2005) Variant histone H2A.Z is globally localized to the promoters of inactive yeast genes and regulates nucleosome positioning. PLOS Biology 3:e384.

Classroom activity (limit 45 minutes)

Pokholok et al.

1. What technique did the authors use to address their research question (you might want to consult the Guillemette paper, which explains this more fully)? Please explain in general terms how this technique works and why it provides information about the chromosomal location of the kinases.

   The process involves crosslinking chromatin proteins to the DNA with formaldehyde, sonicating the complexes to generate short DNA fragments, immunoprecipitating those involving the target protein, reversing the crosslinks, fluorescently labeling the DNA and using it to probe a DNA chip to identify the bounds sites. The location of binding to the chip identifies the location of the DNA in the chromosome.

2. What function do the kinases Hog1, Fus3/Kss1 and Tpk1/Tpk2/Tpk3 play in the cell? What conclusions do the authors draw about the location of these kinases in chromatin and (more importantly) how does the data in the figures allow them to draw these conclusions?

   Hog1 is a signaling kinase regulated by osmotic stress, Fus3/Kss1 are kinases that activate genes in response to yeast mating pheromone and Tpk1/Tpk2/Tpk3 are isoforms of cAMP-activated protein kinase A, which control genes in response to carbon source. Hog1 was localized to promoters but also to adjacent structural genes, Fus3/Kss1 to coding regions, Tpk1 to coding regions of regulated genes in the presence of glucose, and Tpk2 to promoters of ribosomal protein genes. There was no information about Tpk3 binding sites. The figures show increasing binding of labeled DNA to these regions but not to adjacent regions of chromatin.

3. How do the results obtained about the location of the kinases differ among the six kinases tested? Do you think those differences imply that the kinases play different functions in the cell? Why?

   (Sorry this is redundant with the last question!) Hog1 and Tpk2 bind promoters and Hog1 and the others (excepting Tpk3) bind coding regions. The data suggest that Hog1 and Tpk2 have direct roles in regulating transcriptional initiation at the promoter and the others may regulate transcription elongation or chromatin structure within the transcribed regions. So, the data do imply different roles for the kinase based on their interacting with distinct parts of the genes.

Guillemette et al.

4. Compared with the kinases in the previous paper, what is different about the H2A.Z protein that these authors study? Summarize what was known about the distribution of this nonallelic variant histone from previous work. What is the central question that the authors wish to answer?

   H2A.Z is a histone variant so it is present in nucleosomes potentially across the genome. The kinases are not parts of a chromatin structural component and are not expected to broadly bind to chromatin. H2A.Z had been found associated with transcribed regions and to be remodeled in response to transcription but also to bind to heterochromatic regions. The central question here is if there is a specific chromatin location where H2A.Z binds.

5. Where in the chromatin do Guillemette say H2A.Z is generally located? How clearly do their data support this conclusion? How does the rate of transcription of the genes near the binding site affect the occupancy by H2A.Z? Explain how they drew this conclusion.

   They find H2A.Z in or near promoters (near the beginning of coding regions). The ChIP-Chip data show intense binding of the protein near most promoters but less binding to regions of active transcription. These conclusions come directly from the intensity of binding to DNA sequences on the chips that are known to include or be adjacent to the promoter. The resolution of the ChIP-Chip, however, is only about 300 bp so being certain of the location is difficult.

6. How do the authors show the location of H2A.Z with greater precision (the Chip-chip experiments have about 300 bp resolution)? What effect do they show H2A.Z having on nucleosome positioning?

   The authors use the ligation-mediated PCR, which has greater resolution, to further refine the binding location. The technique can not be used on whole genomes; they look at the GAL1 promoter as a model for other H2A.Z-occupied promoters. They find that a nucleosome overlapping the transcription start site shifts about 20 bp in the absence of H2A.Z suggesting that nucleosomes containing the variant histone refine the chromatin structure in the promoter region.

7. The authors show a different distribution of H2A.Z in heterochromatin near the telomeric ends of chromosomes (HZAD genes). How does the distribution differ? What do they propose is the function of H2A.Z in euchromatic genes? What function do they propose for the heterochromatic genes? What two ways do they propose H2A.Z may function in heterochromatin?

   In the HZAD regions H2A.Z is much more broadly distributed, not confined to promoters. Since much of the HZAD region is transcriptionally silenced, the authors attribute the silencing to the broad occupancy by H2A.Z nucleosomes. In euchromatin these nucleosomes "poise" the chromatin structure in a way that allows initiation of transcription to occur. In heterochromatin, by contrast, they suggest that H2A.Z may position nucleosomes so as to block instead of promote initiation of transcription.