

# **Abstracts of the 28th International West Coast Chromatin and Chromosomes Conference**

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## ABSTRACTS / RÉSUMÉS

### Rapid analysis of chromatin composition by quantitative agarose gel electrophoresis

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Previous studies performed on single nucleosomes have indicated an issue of stability at concentrations near or below 5 ng/ $\mu$ L. However, the biologically relevant templates for such analyses are nucleosomal arrays (NAs) and not individual nucleosomes. Our study of chromatin stability was performed using a tandemly repeated 208 bp sea urchin 5S rDNA positioning sequence reconstituted into chromatin using purified histones. To estimate the effect of DNA length on stability, we used either 208–7 or 208–12 NAs. To document histone loss from the long DNA template, standard DNase I footprinting experiments could not be used. Instead we performed a series of quantitative agarose gel electrophoresis experiments that allowed us to evaluate the effect of concentration on nucleosome stability on NA templates. To facilitate and speed up the analysis, we developed a mul-

tipole loading method that allows 4 or 5 samples to be analyzed simultaneously. After calibrating the multiple loading method, we analyzed NAs at concentrations ranging from 20 to 0.625 ng/ $\mu$ L. We observed that histone loss starts to occur between 5 and 10 ng/ $\mu$ L. To investigate the mechanism of histone loss, we performed chromatin precipitation assays in the presence of 10–20 mmol/L MgCl<sub>2</sub>, followed by TCA precipitation and SDS–PAGE analysis. The results indicate that histones appear to be displaced for the DNA templates as octamers. Experiments are on the way to assess whether the octamers at the end of the linear NAs are more sensitive than those located towards the center of the array. We are also investigating the effect of histone tails and histone modifications on actual NA stability.

### DNA-mediated Sir3 silencing

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The current model describing Sir3 silencing describes its mechanism of repression spreading strictly as a histone-tail-mediated phenomenon. However, we have recently shown that Sir3 is capable of binding DNA in a sequence-independent and cooperative manner. The actual affinity for DNA templates is lower than that observed with chromatin templates. Our initial observations indicated that Sir3 binding to chromatin templates left the linker DNA in nucleosomal arrays highly accessible. This result indicates that Sir3 preferentially interacts with DNA that is directly in contact or in close proximity to the core histones. We have investigated Sir3 DNA-binding properties using the well characterized 5S rDNA 208–12 DNA fragment and found the binding to be cooperative. Several DNA sequences displaying various degrees of curvature were used as templates and characterized by EMSA. Sir3–DNA complexes formed complexes of various sizes that were seen as smears at molar ratios ( $r^{\text{Sir3}}$ ) (Sir3/208 bp of DNA) between 2 and 5, indicating heterogeneity. By monitoring half-depletion, we found that

DNA fragments with the lowest amounts of curvature were preferred targets for Sir3 binding. This result somewhat contradicts our initial assessment of a Sir3 binding preference for linker DNA. To determine Sir3's location within a chromatin template, we used a 4-way junction, supposed to mimic nucleosomal entry–exit DNA. Our results strongly suggest DNA conformation to be a poor substrate, indicating that Sir3 recruitment occurs either at the linker or nucleosomal DNA away from the entry–exit point. Finally, we tested DNA binding under increasing Sir3 molarity at various Sir3/DNA molar ratios. A well defined complex was observed at higher molarity ( $r^{\text{Sir3}} \sim 8$ ). The results suggest that Sir3's DNA binding properties are affected by changes in local concentration, possibly through the formation of Sir3 multimers that would possess several DNA binding sites. We believe that these observations, taken together with similar behavior seen on a longer DNA template, strongly suggest a DNA-dependent mechanism for Sir3 initial nucleating and spreading of silencing.