Meeting Report

The post-genomic era: workshop on chromatin immunoprecipitation-related techniques

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Regina Elena Cancer Institute. Rome 11–12 November 2006

The Rome Oncogenomic Center (ROC), together with AIRC, has organized the 'First ROC international workshop and practical course on chromatin immunoprecipitation related techniques'. The workshop has involved a practical course focused on current methods for the analysis, on a genome-wide scale, of chromatin remodeling and molecular complexes identification *in vivo*. During the course, the students (36) have learned how to address these topics from a standpoint and how this field has progressed in the last years from a scientific, technological and bioinformatic point of view.

Genome-Wide Tiling, CpG Island and Custom-Made Arrays: Different Strategies for the Same Purpose

As documented in several talks, a great effort has been devoted in the last 2 years to develop microarray technologies for understanding protein–DNA binding events (ChIP-on-chip). Different technologies are used by different groups. By analyzing genome-wide target site profiling of several transcription factors through the whole-genome tiling array NimbleGen platform, Henk Stunnenberg (University of Radboud, Nijmegen, The Netherlands) has shown how TFs regulate their target genes mostly from distant DNA regions rather than proximal promoters. Motif searches revealed that a rather large number of the *in vivo* target sites did not contain the expected *cis*-acting sequence, indicating that transcriptional regulation frequently involves crosstalk with other factors. Similar results have been reached by Myles Brown (DFCI/HMS/HSPH, Boston, USA). To identify the complete set of steroid receptor regulatory sites, he has undertaken an unbiased approach combining ChIP with Affimetrix oligo-nucleotide microarrays tiled at high density across the entire human genome. He coined the term *CISTROME* to define the set of *cis*-regulatory targets of *trans*-acting factors across the entire genome. With this technology he has identified 3365 ER target sites, most of them in regions previously unrecognized as *cis*-regulatory elements, suggesting that studies focusing only on promoter regions are insufficient. In contrast, he showed that RNA PolII is significantly biased to promoter-

proximal regions and concluded that the communication between the transcription factors and the basal transcription machinery is often mediated at long distances. To gain an unbiased view of *in vivo* NF-Y binding, Roberto Mantovani (ROC, Milan, Italy) performed ChIP-on-chip experiments on the chromosome 21 tiling array Nimblegen platform and found 400–600 NF-Y binding sites. Only 20% of the identified sites reside in the promoters. The vast majority of the sites are contained within transcribed regions (50%), with the remaining 30% located far from annotated genes. One type of array suitable for Chip-on-chip takes advantage of the strong association between CpG islands (CGIs) and gene regulatory regions. Linda Penn (University of Toronto, Canada) has obtained 20736 clones from a CGI Library and used these to construct CGI arrays containing 9595 distinct genomic loci. Using these slides to study Myc target genes, she has optimized key parameters for the ChIP-on-chip assay. She observed that the dye-swap, as well as the combination of sample *versus* input rather than sample *versus* negative control, does not play an important role in the result outcomes. Conversely, antibody quality, array batch, amplification and hybridization need to be tightly controlled. She conducted a large-scale comparison of different algorithms for ChIP-on-chip data analysis, reporting that part of the data interpretation depends on the *in silico* analysis. The contribution of Shirley Liu (DFCI/HSPH, Boston, USA) was specifically focused on the *in silico* analysis of ChIP-on-chip results. She developed a series of algorithms for the analysis of ChIP-on-chip on genome tiling microarrays, including (a) microarray blob remover (MBR) to filter probes in blob defects on the array, (b) an algorithm for eXtreme fast MApping of Nucleotide (XMAN) probes to the genome, (c) a model-based analysis of tiling arrays (MAT) that models probe baseline behavior from probe sequence and genome copy numbers, and (d) a web *cis*-element annotation system (CEAS) for a comprehensive annotation of TF-bound ChIP regions in the genome. Using ChIP-on-chip technology, Xavier Gidrol (CEA, Evry, France) demonstrated that E2F transcription factors are able to

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regulate various cellular processes and are subjected to a functional plasticity during differentiation of human keratinocytes. He used a DNA microarray containing promoter regions of 12 000 human genes (−800/+200 bp relative to the start site). In order to map the replication time zones of the mouse, Itamar Simon (University of Hebrew, Jerusalem, Israel) isolated newly replicated DNA at distinct time points during S phase using a synchronization methodology that he called 'baby machine'. DNA replication at each time point was detected by hybridization to high-density genomic microarrays. He observed that housekeeping genes as well as genes involved in apoptosis and stress responses are replicated early, while tissue-specific genes are replicated late. In order to identify potential correlation between the stress–response and the nuclear location of ERp57/GRP58, Silvia Chicchiarelli (University of Rome, Italy), one of the three selected speakers in this section, cloned the immunoprecipitated DNA fragments and localized them in the human genome. To study how Stat2 (the IFN α -specific component of ISGF3) modulates transcription, Barbara Testoni (ROC, Rome, Italy) built an oligo-based array containing 113 putative IFN α target promoter regions and performed ChIP-on-chip experiments with anti-Stat2 and the activated form of Stat2 antibodies before and after IFN α treatment in hepatoma cells. This allowed her to describe a scenario of Stat2 recruitment to ISG promoters, which greatly extends previous knowledge. To validate the ChIP-on-chip data, she utilized the Applied Biosystems real-time PCR cards to customize an expression assay containing 47 ISGs. With the aim of identifying a specific transcriptional signature for gain-of-function of mutant p53 proteins, Giulia Fontemaggi (ROC, Rome, Italy) described an oligonucleotide array for ChIP-on-chip, enclosing 152 putative mutant p53 targets.

Evaluation of Epigenetic events *In Vivo*

Henk Stunnenberg, using a modification of the ChIP assay to precipitate methylated DNA, performed DNA methylation profiling (MeDIP) and observed that promoters of actively transcribed genes are largely unmethylated, whereas the coding regions of transcribed genes are hypermethylated. Chiara Lanza (Dulbecco Telethon Institute, Naples, Italy), using 3C technology, clearly demonstrated that the analysis of the epigenome structure also concerns the role of *cis–trans* interactions between regulatory elements and their topological organization in the nucleus. The epigenetic rules governing transcription factor binding in eukaryotic genomes are still not clear. Using quantitative chromatin immunoprecipitation (qChIP), Bruno Amati (IFOM, Milan, Italy) found that Myc preferentially binds chromatin enriched for lysines methylation of histone H3 K4 and K79 and acetylation of several H3 and

H4 lysines. Myc binding negatively correlated with H4 K16 acetylation and repressive marks such as tri-methylation of H3 K27 and induced further histone modifications. Similar results were presented by the selected speaker of this session, Aymone Gurtner (ROC, Rome, Italy). She showed that NF-Y colocalizes with acetylated histone H3 and nascent RNA. NF-7 recruits p300 onto target promoters and this correlates with H3 and H4 histone hyperacetylation and active transcription of NF-Y target genes.

Transcriptional Regulation in the Chromatin Environment

Steven Johnsen (EMBL, Heidelberg, Germany) addressed the issue of the dynamics of estrogen receptor transcriptional complex formation in response to estrogen. He provided evidence that LDB1, a cofactor in the regulation of estrogen receptor-dependent transcriptional initiation, is coexpressed with the estrogen receptor as assessed on a tissue microarray containing 1400 breast cancer samples scored for LDB1 expression. Steven Buratosky (HMSD, Boston, USA) has applied ChIP technology to study mRNA processing. He has established a model in which changing phosphorylation patterns of RNA PolII results in the recruitment of different mRNA processing complexes at different points of the transcription cycle. Irene Bozzoni (ROC, Rome, Italy) has studied mRNA processing and has demonstrated that transcription by RNA PolII and subsequent processing of primary transcripts are connected events. Marco Bianchi (University of Milan, Italy) has proposed a revision of the enhanceosome concept in a dynamic framework. Indeed, using FRAP and FLIP technologies, coupled with ChIP and transactivation assays, he showed that local nucleoplasmic concentration of NF- κ B changes on a timescale of seconds and directly impacts on promoter function.

Concluding Remarks

A comprehensive understanding of where regulatory proteins interact with the genome *in vivo* will greatly increase our knowledge on transcriptional mechanisms and epigenetic events. This meeting has clearly indicated how ChIP coupled with genome DNA microarrays will allow the identification of the entire spectrum of *in vivo* DNA binding sites for any given protein and/or protein complex.

Acknowledgements. We thank all the participants for their valuable contributions. A special thank goes to the chairmen and the instructors of the 'first ROC international practical course'. Financial support by AIRC is greatly acknowledged.