Preface

Organisms are defined by their genetic code – DNA. A detailed knowledge of the mechanisms that duplicate, repair and decode the genetic information is fundamental to our understanding of life itself. Inside the nucleus, DNA is associated with proteins to make chromatin, which forms the template for function. The processes that control chromatin function are presently mostly described in terms of individual DNA–protein interactions.

In reality, however, such interactions are parts of a complex molecular interaction networks that are highly dynamic in time and space. Understanding such complex biological systems requires the unravelling of these networks and catching them in quantitative and predictive models, based on quantitative experiments.

How can a systems biology type of approach be applied to the analysis of the regulation of transcriptional activity? A traditional view of gene expression considers how chromatin structure and transcription factors contribute to the regulation of gene expression by activating RNA synthesis. However, it is becoming increasingly clear that genes do not operate in isolation. Rather, they are components in a network of interactions that orchestrate the use of our genetic information. Although we are still far from understanding the behaviour of these networks, we begin to see the contribution of various aspects. Identifying specific protein–DNA interactions provides a snapshot of gene regulation. Given the transient nature of most cell physiological states, the resulting picture highlights parts of the process of how genes are controlled and regulated. The precise characterization of protein–DNA interactions, the characterization of DNA binding proteins respectively, reveals the components involved in the different steps of gene regulation. Those analyses result in a time-dependent resolution of interactions under a variety of physiological conditions. Knowing the time period during which a regulatory protein is bound to DNA provides insights into the underlying biochemical mechanisms.

The challenge we face at present is to understand how the different systems regulating each of these complex activities can contribute to the global regulation of gene expression. At this stage, the pressing question is how to meet this challenge.

Gene promoters bind numerous transcription factors, which are components of regulatory networks. Their synthesis, activation, modification, cellu-

lar localization and turnover are controlled by components of the regulatory network.

A change in gene expression can be obtained by several means: a binding site can become non-functional by mutation, by alternative DNA methylation and histone modification, it can acquire a different function by the same mechanism, or can arise de novo. Even more subtle changes (relative affinity) can result in a different expression pattern of orthologous genes in two tissues, cell lines or species. In conclusion, paralogous genes can acquire different functions by changing their expression pattern or transcriptional responsiveness, whereas their coding sequences can remain identical.

In the field of eukaryotic gene expression, chromatin function and epigenetics we are just at the very beginning of thinking in terms of interaction networks. Our knowledge about gene regulation is increasing rapidly. It is timely to combine the parameter about the chromatin structure and modification, with the genetic information, e.g. methylation, DNA mutations with protein–DNA interaction data, protein network information and affinity data of those interactions.

This book focuses on the fascinating possibilities that emerged during the last years to study protein–DNA interactions in vitro. As an essential part of transcriptional activity during development and in tumorgenesis a separate chapter describing the analysis of DNA modification especially methylation of cytosines at their carbon-5 position was included. Besides the established techniques to characterize protein–DNA interactions by surface plasmon resonance, footprinting techniques and electro-mobility shift assays are still widely used methods. The organism's proteome is a dynamic entity containing thousands of elements involved in numerous intricate networking processes that are related to the developmental stages of the life cycle and to virtually every process in the living cell. A number of methods have been developed to study protein binding to nearly all possible target regions within a genome. To give an insight into those techniques a microarray based method is included. Additionally, a chapter describing in detail the potential of mass spectrometry is included.

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