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Review

Mass spectrometry-based identification of proteins interacting with nucleic acids

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ABSTRACT

The identification of the regulatory proteins that control DNA transcription as well as RNA stability and translation represents a key step in the comprehension of gene expression regulation. Those proteins can be purified by DNA- or RNA-affinity chromatography, followed by identification by mass spectrometry. Although very simple in the concept, this represents a real technological challenge due to the low abundance of regulatory proteins compared to the highly abundant proteins binding to nucleic acids in a nonsequence-specific manner. Here we review the different strategies that have been set up to reach this purpose, discussing the key parameters that should be considered to increase the chances of success. Typically, two categories of biological questions can be distinguished: the identification of proteins that specifically interact with a precisely defined binding site, mostly addressed by quantitative mass spectrometry, and the identification in a non-comparative manner of the protein complexes recruited by a poorly characterized long regulatory region of nucleic acids. Finally, beside the numerous studies devoted to *in vitro*-assembled nucleic acid–protein complexes, the scarce data reported on proteomic analyses of *in vivo*-assembled complexes are described, with a special emphasis on the associated challenges.

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1. Introduction

Understanding gene expression represents a major key to decipher most fundamental processes in cell biology. It is now largely accepted that gene expression regulation is a complex cellular mechanism integrating several levels of control including epigenetics, transcription and translation. Both transcriptional and translational controls depend on interactions with proteins and with regulatory non-coding RNAs, this latter type of interaction being pointed out more recently. The identification of the regulatory proteins that control DNA transcription or RNA stability and translation represents thus a key step in the comprehension of those processes, but constitutes a very difficult task. For this goal, affinity chromatography strategies have been developed, based on a theoretically simple concept. In the first step, a nucleic acid (NA) sequence of interest, either RNA or DNA, is used as a bait, immobilized on a chromatographic support, to capture NA-interacting proteins contained in a protein extract. Once NA/protein complexes are formed, the proteins or protein/NA complexes are eluted and the second step consists of the identification of captured proteins by mass spectrometry.

In this review, we will discuss the different strategies that have been set up to reach this purpose, first in the case of DNA-interacting proteins, and secondly when RNA is used to capture RNA-interacting proteins. The different biological properties of DNA and RNA are important to consider in the design of DNA- or RNA-affinity chromatography features and will therefore be discussed separately for the two types of nucleic acids. However, in both types of NA-interacting protein complexes, the chromatography eluates consist of complex protein mixtures containing low abundant sequence-specific interacting proteins and highly abundant proteins that interact in a non-specific manner with those nucleic acids. As the mass spectrometry-based identification of the specific partners represents a similar challenge for DNA- and RNA-affinity purified proteins, this step will be discussed simultaneously for both nucleic acids interacting proteins.

2. DNA-affinity purification

2.1. *History of DNA-affinity: From biased to -omics methods…*

Selection and modulation of gene transcription in response to environmental changes or developmental signals depend on the coordinated influence of transcriptional regulator interactions with *cis*-regulatory sequences and chromatin modifications [1]. Among transcriptional regulators, one can distinguish at least two categories: those that directly bind to DNA *cis*-regulatory sequences, called transcription factors (TFs), and the co-regulators interacting indirectly with DNA sequences through dynamic interactions with transcription factors. While the general TFs (GTFs) are necessary for transcription to occur, and usually belong to the large pre-initiation complex, the various specific TFs, often activated in response to intra- or extracellular cues, bind to specific binding sequences that can be located in the close proximity of the transcription start site (promoter region) or at long distances (enhancers) [2,3]. The latter can then recruit the basal transcriptional machinery or play on chromatin architecture and nuclear 3D organization through diverse enzymatic activities at the origin of epigenetic modifications [4]. The main difficulty in the study of gene expression regulation comes from the fact that the presence of a binding site for a specific TF does not ensure that this factor actually binds to this site as this also depends on the genomic and protein context surrounding the DNA site, on the abundance, translocation and/or activation of the TF (by post-transcriptional modifications for instance) and on the chromatin status of the loci. Moreover, the precise spatiotemporal modulation of gene expression is due to complex and regulated networks of protein interactions taking part on several binding sites at the level of the promoter or other *cis*-regulatory sequences. The elucidation of these specific interactomes is of major importance to better understand gene expression regulation.

Various experimental methods to assay protein–DNA interactions exist, but many of these methods are based on the fact

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