Approaches to the Analysis of Gene Expression Using mRNA

A Technical Overview

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Abstract

Messenger RNA is the blueprint for all proteins expressed within living systems. Therefore, the study of mRNA expression within normal and diseased tissues is central to our understanding of biological systems. However, blueprints, in themselves, perform no function unless they are used to produce the material for which they code. Spurious results may frequently result from poorly designed or inappropriate studies. This review seeks to highlight both the pitfalls and the promise of various approaches to the analysis of mRNA in different systems and to place these studies in the wider context of research approaches aimed at understanding the function of living systems. The various techniques for the analysis of mRNA are discussed, with particular reference to their potential uses and problems and relevant examples are cited from the literature. It is hoped that this overview of the uses of analytical approaches will allow both the novice researcher and the more experienced scientist to better structure research approaches.

Index Entries: mRNA; gene expression; review, PCR; technique.

1. Introduction

This review deals with techniques of mRNA analysis, both qualitative and quantitative. The technical details for the majority of the techniques described in this article are drawn from the series Methods in Molecular Biology. In this overview I have attempted to highlight some of the fundamentals that researchers need to understand in approaching this area. This review is by no means exhaustive but the aim is to provide an overview of basic techniques that can be applied to the analysis of gene expression both in cell lines and in tissue/tumor biopsies. Detailed descriptions of the practical background of a wide range of molecular techniques can be found in most molecular biology texts. Further protocols can be found in the following volumes which cover specifically the areas of differential display (1), PRINS and polymerase chain reaction (PCR) in situ (2), and basic PCR and mRNA protocols (3, 4). Further protocols are also available on the World Wide Web (e.g., 5).

As with any such research approach, it is imperative that the researcher have a clear goal in mind at the outset of the experimental procedure and that the techniques selected to achieve that goal are appropriate. There are therefore a few basic principles that bear stating at this stage, prior to the examination of the techniques available in this area.

The most significant principle, commonly overlooked by those researching mRNA expression, is that although proteins are effectors, mRNA is a blueprint. In examining the expression of mRNA molecules one gains insight into the regulation of expression and stability of the mRNA species under examination and extrapolates this into an understanding of the regulation of the protein derived from this mRNA blueprint. It is important that this extrapolation is made with care. As with any blueprint, the existence of the blueprint does not prove the material for which it codes has been produced and, conversely, failure to detect the
It is therefore essential to link mRNA analyses with analyses of protein expression and function.

In addition, owing to the extreme sensitivity of modern analytical techniques, especially PCR, it is possible to detect extremely low copy numbers of mRNA species within cell populations, and the relative importance of these molecules must be related to the copy numbers detected. Indeed, with such sensitive methods available, the possibility of stromal or other sources of contamination must be borne in mind. In common with all analytical techniques, it is imperative that the composition of the tissue under examination be understood before correct interpretation of results can be carried out. This knowledge is particularly important in solution phase studies where mRNA is extracted from entire tissues and the possibility of incorrectly assigning expression to, for example, tumor rather than stromal components exists.

So why use mRNA analyses at all? There are a number of significant advantages to mRNA analyses over conventional protein analyses, affecting sensitivity, ease of execution, sample throughput, and evaluation of expression levels.

The advent of the reverse transcriptase-polymerase chain reaction (RT-PCR) increased sensitivity of mRNA detection significantly when compared with Northern blots or RNase protection assays (RPA). The ability to detect copies of mRNA in the low hundreds (attomolar \(10^{-21}\)M range \([6]\)) meant that the amounts of tissue required could be reduced significantly, or, conversely, the number of products screened could be increased. Using modern PCR systems it is now feasible to PCR up to 384 reactions in a 2–3 h incubation, providing sufficient throughput for most screening applications. Furthermore, the use of reverse transcription of mRNA to stabilize material for long-term storage is an additional advantage of this approach \((7–8)\).

Although detecting the expression of mRNA transcripts is an important first step in any investigation, questions relating to level of expression, regulation, and location (tumor or stroma, proliferative or nonproliferative cells) are immediately raised. mRNA studies are extremely powerful for the detection of dynamic changes in expression within and between cell populations. Quantification of expression (either with respect to an internal control or with full molar quantification) can be performed using Northern blotting, RNase protection assays, or, increasingly, RT-PCR using either real time approaches or PCR mimics \((7–11)\). These methods can be applied to the study of differential expression between tissues and induction or clearance of mRNA species. Although care is required in the individual experimental design, mRNA studies give an immediate and sensitive method for the investigation of the dynamics of gene expression.

Increasingly, conventional targeted strategies for comparing expression between tissues are being expanded to provide global comparisons of gene expression between, for example, neoplastic and non-neoplastic tissues. Formally the domain of those able to perform complex mRNA subtraction library analyses, the recent development of techniques relating to DNA chips, serial analysis of gene expression (SAGE), and differential display (displaying the mRNA profiles from separate sources and noting differences) has been widely applied to the study of tumor-specific gene regulation. Such techniques are reliant on careful validation of the approach taken by repeat experiments using targeted sequences (e.g., PCR or RPA). However, these techniques can be immensely powerful in the detection of novel or known genes that are up- or down-regulated in transformed tissues \((12–13)\).

All the preceding techniques can be compromised by the fact that all tissues are not homogeneous in their cellular composition. The presence of blood vessels, inflammatory cells, stromal components, and a mixture of tumor and normal tissue cells can complicate analyses. It is imperative, at some stage of the investigation, to determine the tissue source of the mRNA detected in order to avoid ascribing a tumor origin to a stromal protein or mRNA. The ability to use both archival (paraffin-embedded) and fresh (frozen) material has been the major advantage to the