Leading the Way Using Microarray
A More Comprehensive Approach for Discovery of Gene Expression Patterns

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Cells react to various stimuli by modulating their biological functions through selective changes in the activities of their constituent proteins. These changes are rendered by secondary modifications, translocation, or interaction with other molecules or cofactors or by altering a protein’s concentration. The latter event occurs via regulated modifications in the rate of gene transcription, RNA translation, or degradation of RNA or protein. In the premicroarray era, the number of genes that were subject to differential expression was seriously underestimated; this is demonstrated in the study by Stanton et al in this issue of Circulation Research. Using microarray technology, Stanton et al identified more than 700 genes whose expression was altered during myocardial infarction. Expression profiling is not merely a descriptive method; disclosure of the temporal and spatial changes in gene expression provides insight into cellular functions and underlying mechanisms in disease pathogenesis.

DNA microarrays, or gene chips, are usually comprised of micron-range–sized spots of genomic DNA, cDNA, or oligonucleotides arrayed on a glass slide. They are used for a wide scope of applications, including sequencing, detection of mutations or polymorphisms, identification of drug targets, and gene expression profiling (reviewed by Wilgenbus and Lichter). The latter application has gained wide use for monitoring differences in gene expression patterns in normal versus pathological conditions. Different clustering methods have been devised for the management of the large number of data points obtained by this method. One such method, demonstrated by Eisen et al and also used by Stanton et al, relies on clustering of genes according to similarities in their temporal expression patterns. The study of gene clusters based on temporal expression pattern in yeast has revealed that genes with similar functions tend to exist within the same cluster. For example, yeast responded to heat shock by downregulating the expression of 112 genes involved in various aspects of protein synthesis, all of which displayed the same time course of expression. One might use this information as a reference to predict the functions of cells under given conditions or to assign a putative function to an unknown gene. To further interpret their results, Stanton et al used an additional method for analysis of their data. They classified the differentially expressed genes into 7 groups based on their biological functions. Within these groups, they identified nonrandom distribution of expression patterns. This method directly revealed the functions emphasized by the cells in a heart with an area of infarct. Although these functions (including translation and transcription, metabolic changes, cell signaling, proliferation, and changes in cell structure) were previously known to be operative in infarct zones, the identity of the majority of the contributing molecules had remained obscure.

The main question, then, is: what did we learn from the microarray data by Stanton et al that we did not already know? By revealing the identity of a broad spectrum of molecules involved in the different cellular functions, the results allow us some degree of mechanistic speculation. For example, although we previously knew that cells undergoing hypertrophy exhibit an increase in ribosomal content underlying the increase in total protein synthesis, the microarray data revealed additional details. The increase in cellular protein induced by an increase in ribosomes is conceivably catalyzed by the overlapping increase in factors responsible for initiation and elongation of synthesis, such as elongation factor 1-α and eukaryotic initiation factor-5A. It is also plausible that the accompanying increase in total RNA synthesis is affected by the upregulation of RNA polymerase II, transcription factor S-II, basic transcription factors, or another candidate. Increases in factors of a more specialized nature, such as cardiac ankyrin repeat protein, MEF2C, TSC-22, Sox-4, or ATF4, that may be responsible for specific gene transcription, are superimposed. Our recent studies using subtractive hybridization on hearts subjected to pressure overload yielded similar results.

However, one limitation is that the expression profile presented in the study by Stanton et al represents transcripts from several cell types that are undergoing different functional transformations. Among these are cells of the immune system that have migrated to the infarct region and are responsible for an inflammatory response, cardiac myocytes within the ischemic area undergoing apoptosis and necrosis, fibroblasts undergoing proliferation and participating in the formation of scar tissue to replace the infarct, and cardiac myocytes undergoing hypertrophy to compensate for the loss of cells in the infarct area. Although the nature of the regulated genes might help define the parent cell, additional

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experiments to localize the transcripts (ie, using in situ mRNA hybridization) are vital.

Noteworthy is that the authors used 7000 randomly selected cDNA clones from a cardiac ventricular library for generating the microarray as opposed to nonrandomly selected known genes or expressed sequence tags. Although the former approach is biased toward detection of transcripts of higher abundance, it eliminates bias toward the detection of known genes only. Indeed, ≈10% of the genes differentially expressed in the infarct seem to be novel. A related methodological consideration is that the nature of the cDNA sequences identified by microarrays will be constrained by the composition of the library used for their discovery. In the study of infarct hearts, the library used was made from normal ventricular tissue; hence, any de novo–expressed genes will go undetected. Such genes might include those involved in the cell cycle, whose mRNAs are at a very low abundance in normal cardiac myocytes but are known to be reinduced during cell hypertrophy and proliferation. Likewise, genes involved in cellular defense may remain undetectable until immune system cells are recruited to the infarct zone. Indeed, transcripts of the latter 2 categories of genes are underrepresented in the screen. One solution would be to generate a library from a mix of both normal and infarct tissue for a more inclusive screen.

Finally, if the lessons we have learned from yeast can be generalized, then one has to caution against using expression profiling to predict the relative functional importance of genes. Recently, Winzeler et al reported that genes that are induced in yeast growing in minimal medium did not prove more important for growth than ones that are not induced. In fact, only 2 of 8 genes that are essential for yeast growth in minimal medium are upregulated on that occasion. In other words, genes that are not differentially expressed may be of equal functional importance to those that are and should not be disregarded on that basis. Moreover, it is important to remember that some genes may be regulated at the translational rather than the transcriptional level, which would preclude detection by DNA microarrays.

In conclusion, although the data obtained by expression profiling are by no means an endpoint, they provide a strong foundation for hypothesis-driven research about the role and mode of regulation of the identified molecules during the process in question.

References

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